SHOUT
SHARE
IT

874

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

Editorial Staff

E. G. Conklin—*Princeton University.*
Jacques Loeb—*The University of California.*
T. H. Morgan—*Columbia University.*
W. M. Wheeler—*Bussey Institution, Harvard University.*
C. O. Whitman—*The University of Chicago.*
E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

Volume XVI.

WOODS HOLE, MASS.
DECEMBER 1908 TO MAY 1909.

PRESS OF
THE NEW ERA PRINTING COMPANY
LANCASTER, PA

CONTENTS OF VOLUME XVI

No. 1. December, 1908

Child, C. M. *Regulation of Harenactis attenuata in Altered Environment.* 1

Mayer, Alfred G. *On the Use of Carbon Dioxide in Killing Marine Animals* 18

Hegner, R W. *Effects of Removing the Germ-cell Determinants from the Eggs of Some Chrysomelid Beetles. Preliminary Report* 19

No. 2. January, 1909

King, Helen Dean. *Studies on Sex Determination in Amphibians. II.* 27

Mathews, A. P. *The Influence of Some Amino-acids on the Development of Echinoderms* 44

Child, C. M. *Experimental Control of Certain Regulatory Processes in Harenactis attenuata* 47

Lillie, Frank R. *Polarity and Bilaterality of the Annelid Egg. Experiments with Centrifugal Force* 54

Bigelow, R. P. *A New Narcomedusa from the North Atlantic.* 80

No. 3. February, 1909

Patterson, J. Thos. *An Experimental Study on the Development of the Vascular Area of the Chick Blastoderm* 83

Mast, S. O. *The Relations of Didinium nasutum (Stein) with Special Reference to the Feeding Habits and the Function of Trichocysts* 91

Payne, Fernandus. *Some New Types of Chromosome Distribution and their Relation to Sex* 119

No. 4. March, 1909

Payne, Fernandus. *Some New Types of Chromosome Distribution and their Relation to Sex* 153

Hilton, William A. *The Hyo-branchial Apparatus of Typhlotriton spelæus Stejn* 167

MORSE, MAX. *The Autotomy of the Hydranth of Tubularia* 172
BECKWITH, CORA JIPSON. *Preliminary Report on the Early History of the Egg and Embryo of Certain Hydroids* 183
GUYER, MICHAEL F. *On the Sex of Hybrid Birds* 193
WILSON, EDMUND B. *The Female Chromosome Groups in Syromastes and Pyrrochoris* 199
TANNREUTHER, GEO. W. *Observations on the Germ Cells of Hydra*. 205
TANNREUTHER, GEO. W. *Budding in Hydra* 210

NO. 5. APRIL, 1909

FOOT, KATHARINE, AND STROBELL, E. C. *The Nucleoli in the Spermatocytes and Germinal Vesicles of Euschistus variolarius*. 215
STOCKARD, CHARLES R. *Inheritance in the "Walking-stick," Aplopus mayeri* 239
DOWNING, ELLIOT R. *The Connections of the Gonadial Blood Vessels and the Form of the Nephridia in the Arenicolidæ* ... 246
GRAVE, B. H. *Some Observations on the Habits of Pecten dislocatus* 259
MORGAN, T. H. *The Dynamic Factor in Regeneration* 265

NO. 6. MAY, 1909

CHILD, C. M. *The Regulatory Change of Shape in Planaria dorotocephala* 277
SHULL, CHARLES A. *Some Abnormalities and Regeneration of Pleiopods in Cambarus and Other Decapoda* 297
HOLMES, S. J. *Sex Recognition in Cyclops*. 313
RIDDLE, OSCAR. *Our Knowledge of Melanin Color Formation and Its Bearing on the Mendelian Description of Heredity* ... 316

Vol. XVI. *December, 1908.* *No. 1*

BIOLOGICAL BULLETIN

REGULATION OF HARENACTIS ATTENUATA IN ALTERED ENVIRONMENT.

C. M. CHILD.

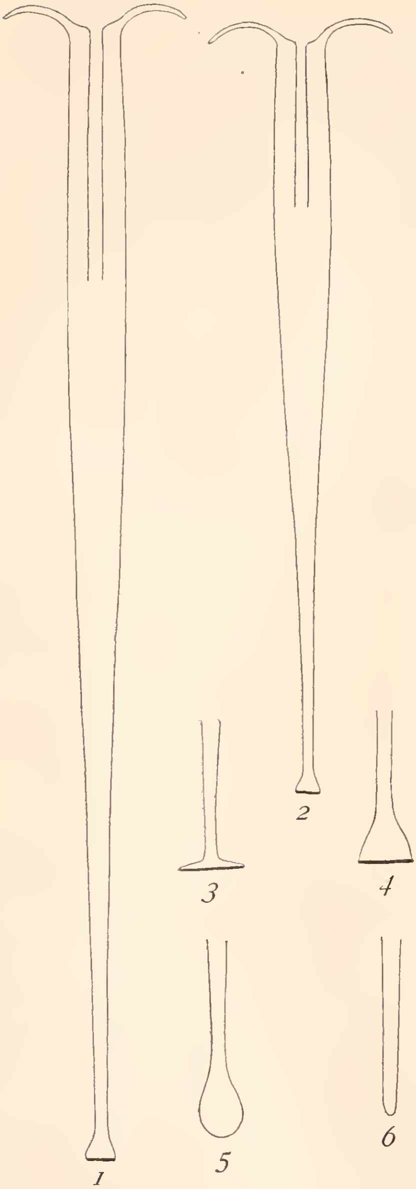
In the course of several months work during the autumn and winter of 1905–6 at the laboratory of the San Diego Marine Biological Association, in La Jolla, California, I had the opportunity of investigating in some detail certain of the regulatory phenomena and conditions determining them in *Harenactis attenuata* (Torrey, '02). Most of my work on this species was concerned with restitution and will be presented elsewhere. Certain of the data obtained, however, concern certain regulatory changes which occur under altered external conditions. Since these are of some interest in themselves and are not immediately related to restitution, they are presented here, apart from the other data.

I. The Usual Form and Habit.

Harenactis attenuata is found in great abundance on the tide flats of False Bay and San Diego Bay, living, as Torrey has stated (Torrey, '02, p. 384), with the column imbedded in fine sand, the axis of the body being perpendicular or nearly so to the surface. When the animal is undisturbed the tentacles, twenty-four in number, extend laterally over the surface of the sand, though very often the tentacles appear as if in two sets, twelve of them, alternating with the other twelve, extending more or less upward and sometimes curved inward, while the other twelve extend laterally and may be curved slightly downward. Often, when the body is well distended the column may protrude a short distance from the sand.

Any strong stimulus causes immediate withdrawal of the oral end from the surface, often for a distance of several inches, and usually more or less complete invagination of the disc and tentacles.

As regards size and shape, the same individual may differ greatly at different times, according to the degree of distension with water and of muscular contraction. In the condition of extreme extension and distension, the larger individuals possess approximately the shape diagrammatically outlined in Fig. 1.[1] Fig. 2 represents a shape which is perhaps nearer the average. In general I have found that the larger individuals are not only absolutely but relatively longer than the smaller. Apparently growth, at least in those later stages of development which I have observed, is chiefly in the longitudinal direction. But the length differs considerably according to external conditions. In certain regions I found the animals living in soft sand which was underlaid at a depth of 20–25 cm. by a layer of broken shells. Under these conditions the



Figs. 1–6.

---

[1] Figs. 1–6 are somewhat less than one half natural size; other figures are about three fourths natural size.

length of the animals was never much greater than the depth of the layer of sand. On the other hand, when they live in a sufficiently thick layer of sand they may attain a length of 40–50 cm. The greatest diameter of the column near the oral end is 15–25 mm. From this region the column gradually tapers to an extremely attenuated proximal portion, which differs greatly in length at different times and in different individuals.

When the animals are removed from their burrows the aboral end is usually more or less button-shaped or disc-shaped (Figs. 1–4) and firmly attached to a bit of shell, a small stone or other solid object. It is, in short, structurally and functionally a foot. In removing the animals from the sand this foot is often lost, since the attenuated region just distal to it is relatively weak. Sometimes, if the foot happens to be attached to some object near the surface — within 5–10 cm. — the attenuated region may be completely absent and the animal may possess something the shape of Fig. 10.

In some cases, however, the foot is not attached to any solid object; under these conditions it may take the forms shown in Figs. 5 and 6 or almost any form intermediate between them. No well defined adhesive surface is developed in these cases, though grains of sand may in some cases adhere to some part of the region. These marked differences, occurring under natural conditions, suggest that the differentiation of a localized adhesive foot is dependent, at least in some degree, upon contact with a solid substratum, a suggestion which is confirmed by the facts cited below. Such forms of the aboral end as those in Figs. 5 and 6 undoubtedly result from the failure of this region to come into contact with a large solid body.

The diameter of the disc is scarcely greater than the greatest diameter of the column; the tentacles of large individuals, when fully extended may reach a length of 40 mm.

The circular muscles of the body-wall are strongly developed throughout and contraction is often unequal in different parts of the body, so that marked constrictions occur. The mesenteries bear powerful longitudinal retractor muscles, which are inserted distally near the free end of the œsophagus. Contraction of these muscles invaginates the disc and tentacles (Fig. 7) and in extreme cases reduces the length of the whole body to a small fraction of what it is when fully extended.

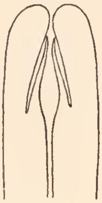


FIG. 7.

In the condition of extreme contraction following some violent stimulus almost all of the water is expelled from the enteron, largely through the cinclides, and the body-wall contracts until the enteric cavity is so reduced that further contraction is practically impossible on account of the bulk of the mesenteries, muscles, filaments, and gonads, if present, which now fill the enteron.

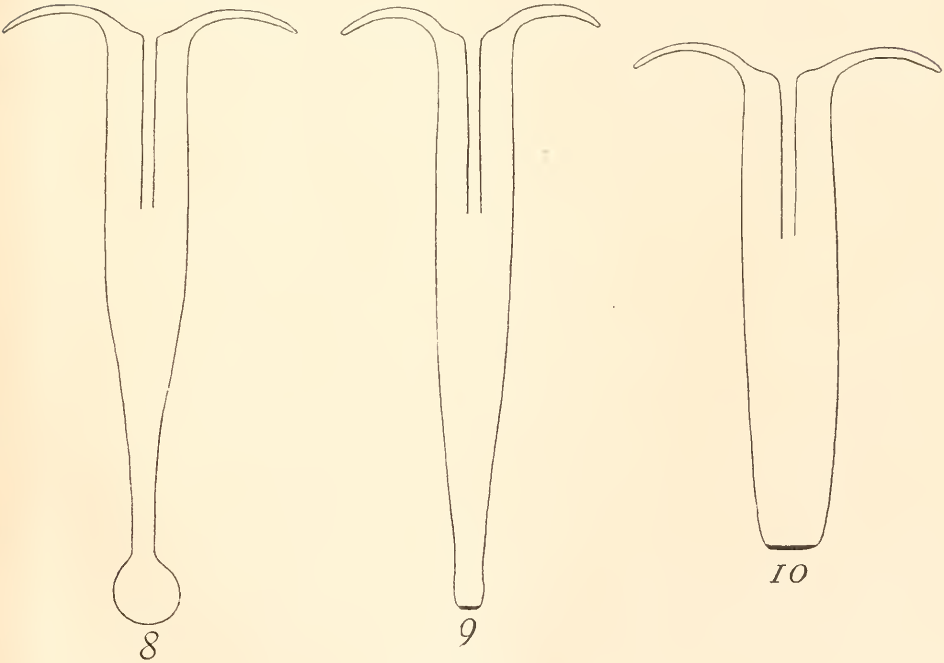
II. REGULATION FOLLOWING REMOVAL FROM SAND.

In the laboratory the animals were kept in dishes, containing water several inches in depth, but without sand. This species is exceedingly hardy and will live for months under these conditions with occasional change of water. In my experiments I kept many specimens under these conditions and without food during four and a half months, and at the end of this time they were apparently in good condition, though smaller than when collected. It is under these conditions that the changes to be described occur.

1. *The Earlier Stages of Regulation.*

As long as the animal is in its burrow in the sand the pressure upon the body-wall of the water in the enteron is supported in large measure by the walls of the burrow, not by the body-wall alone. After removal from sand the body never attains the degree of extension and distension which it may attain in the burrow. As in *Cerianthus æstuarii* (Child, '08), so here, a regulation undoubtedly occurs both as regards the amount of water usually in the enteron, and the ability of the body-wall to sustain the pressure unaided.

The effect of the altered conditions appears first chiefly in a decrease in length and frequently a slight increase in diameter of the column. Figs. 8, 9 and 10 show three common types of the shape of the body after twenty-four hours in water without sand. There is great variation in the shape of both body and foot at this time. In case the foot was originally attached to a bit of shell or other solid body it usually frees itself soon after the animal is brought into a vessel without sand. Afterward it may become attached to the side or bottom of the vessel or may remain unattached. Very commonly when the foot is free, the aboral end of the body takes the shape of Fig. 8, though forms like Fig. 9 often appear. In some cases the attenuated posterior region is entirely absent and the animal is attached by a disc of nearly the same diameter as other parts of the body (Fig. 10), or the aboral end may be free and rounded. These various shapes are merely the visible expression of various states of contraction or extension and change more or less widely in the individual. The most interesting feature is that the body outside of its burrow never in any case, so far as my observations go, extends as completely as in the burrow.



Figs. 8–10.

The animals in water without sand very commonly exhibit negative geotaxis in some slight degree, though never as strongly as *Cerianthus* (Loeb, '91 ; Child, '03, p. 243). No matter how firmly the foot may be attached to the substratum, the animal is not capable of maintaining itself in an erect position in the water, so long as it retains the elongated form (*e. g.*, as in Figs. 8–10). The geotactic reaction usually manifests itself as in *Cerianthus* by the bending of the body which lies on the bottom of the dish, so that the disc lies in the horizontal plane and the axis of the body immediately below it is vertical. The reaction proceeds no further, apparently because the body is incapable of supporting itself erect. This reaction is by no means universal: very frequently it seems to be almost or wholly absent. Occasionally individuals which are strongly contracted longitudinally are found standing almost erect in the water, *i. e.*, the body-wall is capable under these conditions of supporting itself in the water.

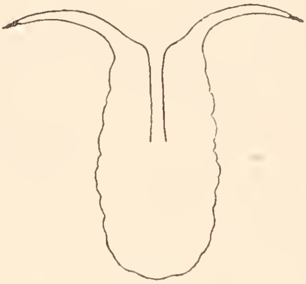


FIG. 11.

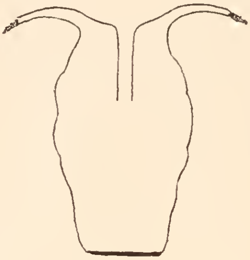


FIG. 12.

The decrease in average length continues from day to day in water without sand, though of course with many fluctuations, as the animal contracts or extends. After a week or two under these conditions the animals usually resemble Figs. 11 and 12 in shape. If the foot is attached, the aboral end is usually a flat disc as in Fig. 12, if it is free, the body is usually a more or less rounded sac without any visible demarcation of the foot-region (Fig. 11). The attached individuals usually stand more or less erect in water, and the unattached lie on their sides. In most cases the free individuals lying on their sides show little or no reaction to gravity, but the absence of the visible reaction is probably due merely to the fact that the stimulus is not sufficient to induce bending of the column in its shortened condition. If these individuals happen to become attached they often become erect.

The body-wall usually shows numerous transverse wrinkles and folds at this stage of regulation, but these gradually disappear with time.

2. *The Later Stages of Regulation.*

Any distinction between the earlier and later stages of this regulation must be more or less arbitrary. The important point, however, is that what is at first merely a condition of partial contraction and capable of rapid alteration under changed conditions, becomes, in consequence of partial atrophy of certain parts and other changes, a relatively permanent form. The transition from the one condition to the other is of course gradual and proceeds at different rates in different parts.

The first directly visible indication of atrophy occurs at the tips of the tentacles. In *Cerianthus* (Child '04*d*, '05) such atrophy of the tips of the tentacles occurs when the internal pressure due to water in the enteron decreases, and as will appear elsewhere, the same process occurs in *Harenactis* under similar conditions. In the present case the tentacle-atrophy is undoubtedly a consequence of the decreased internal water pressure which follows removal from the burrow. Usually the tentacles underwent reduction to about half their original length during the four and a half months of the experiments, though individual differences were of course considerable.

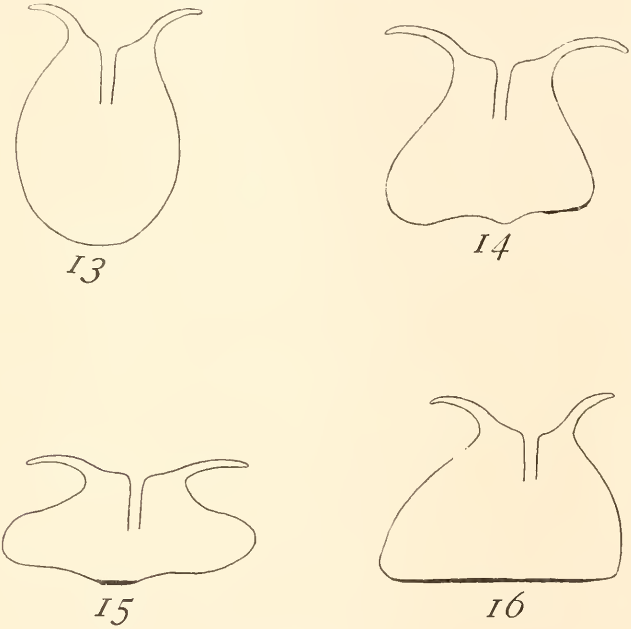
As noted above, the transverse folds and wrinkles of the body-wall gradually disappear. Extensive regulatory changes occur throughout the body-wall. In *Cerianthus* regions of the body-wall which are sharply folded or contracted for any considerable time undergo more or less atrophy (Child, '05, '08) while on the other hand stretched regions grow. The present case is similar: undoubtedly more or less atrophy occurs throughout the body-wall in consequence of the decreased distension, but the atrophy is greatest in those regions where the conditions depart most widely from the original conditions of distension, viz., in the folds. The tissue of these parts is not functioning or is functioning only in slight degree, and its substance probably undergoes resorption in consequence of the demand of other more actively functioning parts for nutrition.

Another marked though gradual change which occurs is in the longitudinal retractor muscles. Contraction and invagination of the oral end is usually rapid and considerable in amount in animals newly removed from their burrows, but as the length of the body decreases this reaction becomes less and less marked. Animals in the condition of Figs. 11 and 12 are usually incapable of complete invagination of the disc, no matter how powerful the stimulus. Usually invagination does not include more than the basal half or perhaps two thirds of the tentacles in such cases, though originally the disc and tentacles could be withdrawn far into the body.

Two factors are probably concerned in this decrease of the power of invagination of the oral end: in the first place, the retractor muscles are undoubtedly undergoing atrophy from disuse, since as the body decreases in length their functional activity is much reduced; and secondly, the enteron is much smaller than it was originally and, as can readily be shown by opening one of these reduced specimens, is almost wholly occupied by the mesenteries with their muscles and filaments, and the gonads, if they are present, leaving but little space for water. Consequently when contraction occurs, decrease in size can occur only to the extent that water is expelled. In these specimens, after the expulsion of the small amount of water present, the body is essentially a sac packed full of tissues and no further contraction is possible unless parts of these are extruded, which does not commonly occur in *Harenactis*.

Some of the different individual shapes after four and a half months of life outside of a burrow are shown diagrammatically in Figs. 13–16. Fig. 13 represents a type very common in my experiments. These specimens have apparently lost all power to attach themselves and the aboral end shows no trace of a distinct foot-region. The whole body is merely a rounded sac with but little power of elongation or of contraction, and invagination of the oral end does not proceed beyond a slightly decreased diameter and increased concavity of the disc. Animals of this shape occur chiefly among those whose attachment to the bottom of the dish has often been disturbed, *e. g.*, by changing the water or by examination for other purposes. I am inclined to believe that many of these individuals have completely lost the power of attaching themselves, though it is possible that the sac-like form of the body is responsible for their failure to attach, since the aboral region usually does not come into contact with the substratum.

Figs. 14 and 15 show other cases, in which the decrease in length is still greater, but in which the aboral end is more flattened and still retains the power of attachment. These cases illustrate the conditions usually found when the attachment to the substratum is less frequently disturbed. Although a flattened aboral region of greater diameter than any other part of the body is present, attachment does not always occur over the whole surface; apparently, however, any part of the surface is capable of serving as the organ of attachment, the animals being attached in some cases by a small region in the middle of the flattened surface (Fig. 15), and in other cases by some part or parts of its margin (Fig. 14). In fact the region of actual attachment may differ at different times in the same individual.



Figs. 13-16.

The form that is in some respects of greatest interest is shown in Fig. 16. In such cases the body is usually attached over more or less of the flattened aboral surface, though occasionally such forms appear without actual attachment, provided the body is broad enough to stand upright on its aboral end. Apparently the mere contact without attachment is sufficient to bring about more or less flattening of the aboral end. In these flattened forms the power of invagination of the oral end is almost entirely absent. Violent stimulation may bring about a slight temporary increase in the flattening, with perhaps some depression of the oral region which does not amount to actual invagination.

The interesting feature in these cases is that the originally greatly elongated, attenuated body has acquired a form almost exactly similar to that of those actinians which are normally sessile on the surfaces of rocks, etc. The ability to retract the oral end has been almost or wholly lost, but from certain observations toward the conclusion of my experiments, it seems not impossible that in the further course of regulation this power may be regained to a greater or less extent. These observations were simply that many individuals seemed to possess a greater power of retraction of the oral end after four and a half than after three and a half months. If this difference is real it may be due either to the regulation of the longitudinal muscles so that further contraction is possible, or to regulatory decrease in the bulk of mesenteries, filaments, etc., or to both factors. The volume of the retractor muscles very evidently decreases during the regulation, and it is extremely probable that the other parts, being folded and packed together so much more closely than originally, undergo more or less atrophy. In short, it appears almost certain, though the length of time covered by my experiments is insufficient for a demonstration, that *Harenactis attenuata* may, in altered environment, acquire most of the characteristics of those actinians which are normally sessile on surfaces exposed to the water.

Individuals removed from their burrows and placed upon the surface of the sand usually succeed in working their way back into it by means of the attenuated aboral end which acquires the form of Fig. 6 under these conditions. After the regulation has occurred, however, they appear to be absolutely incapable of extending sufficiently to insert the aboral end in the sand, or else they have lost their earlier method of reaction. Whichever the case, animals like Figs. 13–16 have never been observed to enter the sand and resume their original habit, after being placed on the surface of sand or even being imbedded in it. In the latter case they usually work their way out of the sand in a few hours and lie on its surface. It seems probable, however, that if they could be kept imbedded in the sand for a considerable length of time, more or less, perhaps complete return to the original form and habit might occur. Further experiment is necessary to determine this point.

3. *The "Foot" of Harenactis.*

In the figures showing the foot, the region actually attached to the substratum is indicated by a heavier line. As the figures show at once, this region is extremely variable in extent: in Figs. 5 and 6 there is no distinct region of attachment, in Figs. 1 and 2 the region of attachment is a very small area, in Figs. 3 and 4 it is somewhat larger, in Figs. 10 and 12 it is a disc almost equal in diameter to the other regions of the column, and finally in Fig. 16 its diameter is much greater than that of any other part of the body. In Figs. 14 and 15 only a small part of the flattened aboral surface is attached, the region of attachment being in the one case in the middle and in the other on the margin. In Fig. 13 there is no trace of an adhesive region, and individuals of this sort which have been unattached during several weeks appear to be unable to attach themselves again, even if placed with the aboral end in contact, though it is probable that they might again acquire the power of attachment after a time.

The foot-region is not markedly different anatomically from the other parts of the body-wall. When an attached region is freed and examined it usually appears somewhat more transparent than other regions, but otherwise essentially similar to them. Nevertheless the region of the body within which attachment may occur is more or less sharply limited. Individuals which lie on their sides in the dishes do not become attached unless the region in contact with the dish is near the aboral end, in which case they may sometimes attach themselves.

We may conclude then that a more or less well defined aboral region of the body is usually capable of reacting to contact with solid objects by adhesion. Only those parts of this surface which are or have very lately been in actual contact are adhesive at any given time.

But the limits of the region within which reaction to contact by adhesion is possible may vary very considerably, as the observations above cited show. At the time of removal from the natural habitat the region of attachment is always limited, so far as my observations on several hundred individuals go, to a relatively very small area at the aboral end (Figs. 1–4). Sometimes after a day or two in the laboratory animals are found attached by a considerably broader area (Fig. 10), but I have never seen the slightest indication that regions still farther from the aboral pole possessed at this time the power to react in this manner, though I have often observed animals with these regions in contact. But after several months in shallow water without sand individuals like Figs. 14, 15 and 16 are of frequent occurrence. In these any portion of the broad flattened aboral region is capable of reacting to contact by adhesion and sometimes, as in Fig. 16, the whole region is involved. In Fig. 16 the radius of this foot-region is almost equal to the length of the column oral to it: if all parts of the body-wall have undergone atrophy in equal degree then the foot-region as shown in Fig. 16 consists of what was originally the aboral half more or less of the column. It is of course impossible to determine whether all parts of the body-wall have undergone atrophy in equal degree, but that point aside, it is sufficiently evident that the extent of the foot-region is very different in Figs. 1 and 16. If the regions of the body oral to the small disc of attachment in Fig. 1 were definitely specified as regions capable of attachment, we should expect to find objects adhering to them, at least occasionally, for they frequently come into contact with solid objects in the walls of the burrow. In some cases I have observed objects adhering to the oral as well as to the aboral surface of the disc of attachment, but never to regions oral to this.

In short the differences in extent of the foot-region in extreme cases like Figs. 1 and 16 show that the extent of the region capable of the adhesive reaction may be altered very considerably by external conditions. It is probable that the adhesion of a given region increases the physiological specification of adjoining regions in the same direction, so that if these come into contact with the substratum they may adhere, and in their turn affect other adjoining regions and so on. Under the usual conditions of life other reactions, and especially the extension of the body to the surface of the sand interfere with the attachment of large regions, aborally, so that the disc of attachment usually remains very small. But when we eliminate the factor of extension and induce instead a shortening of the body, as we do in keeping the animals in water without sand, the conditions are favorable, if the aboral end is in contact at all, for bringing larger and larger areas, into contact and an extension of the actual adhesive region is the result. How far such extension might be carried it is impossible to say.

On the other hand, cases like that represented in Fig. 13 seem to indicate that the aboral region may lose the adhesive reaction if it is forced to remain unattached or is detached as often as it attaches itself. Individuals like this which had been unattached for several weeks or more failed to attach themselves even when the aboral region was brought into contact with the bottom of the dish and kept there by propping the animal up in an erect position. Such evidence is of course negative, but I believe it permits at least the conclusion that long continued absence of contact decreases the ability of the aboral end to react to contact by adhesion.

4. *Conclusion.*

The transformation within a relatively short time of the extremely elongated, burrowing type of body into the short, broad form characteristic of the sessile actinians is of interest in that it indicates the rôle which external factors play in determining the actual shape, and to a considerable extent the size relations of different parts. The elongated form is apparently impossible without the support afforded by the sand in the burrow. The chief factor in bringing about the change in form when the animals are removed from the sand is probably a change in distension of the body by water in the enteron. In animals kept without sand the enteron never contains anything like the amount of water that is present when they are fully extended in the burrow. In the burrow the whole surface of the body-wall except the oral end is supported by the walls of the burrow, and so does not support the pressure of the enteric water. The oral region and the tentacles are evidently adapted to support a high pressure. Outside the burrow, however, the body-wall supports the whole pressure and one of several results is to be expected: first, distention may increase to a point where pressure of the body-wall upon its contents is so great that no more water can enter or where water passes out as rapidly as it enters; second, rupture of the body-wall may occur; third, a regulatory increase in strength or regulatory growth of the body-wall may occur; fourth, the entrance of water may be regulated by some mechanism of correlation. There is every indication that such a mechanism is present in the actinians, for distension of the body-wall beyond a certain point undoubtedly results in regulation in some degree of the quantity of water entering the body, or else in opening the outlets. Evidently such regulation occurs in *Harenactis* for the internal pressure in animals outside the burrows never approaches that which frequently exists within the burrow. The decrease in length of the body, the atrophy of the tips of the tentacles and the partial atrophy of the body-wall, the shortening of the retractor muscles and mesenteries are all very evidently consequences of the decrease in distension. As in *Cerianthus* (Child, '04, *a*, *b*, *c*, *d*, '05, '08), so here, the body-wall, the tentacles, the muscles, and mesenteries cannot maintain their form except under a certain degree of tension or stretching. When this decreases atrophy occurs to a greater or less extent and more or less change in shape may result according to conditions.

The broad foot-region of some individuals (Fig. 16) is, I believe, merely an indirect result of the change in shape which makes contact possible over a large area. Undoubtedly a broad foot-region would appear normally or occasionally in *Harenactis* if conditions existed within the burrow which permitted continued contact between a large aboral area and a solid substratum.

The transformation from the "normal" elongated form to the short, broad "sessile" shape is very evidently a regulatory reaction to altered conditions. But the question as to why the elongated form is characteristic of animals in nature, *i. e.*, in burrows, has not been considered. Evidently this shape is not independent of external conditions, for when we alter the conditions the shape changes. But why should not the animal take the form of a rounded sac or a flattened disc in the sand as well as elsewhere? It is of course easy to say that the form is inherited, but besides being no explanation, this is obviously not true in this case.

In my opinion it is impossible to account for the shape of the adult *Harenactis* without considering its behavior. In the first place, the burrows of the species are almost invariably vertical or nearly so, and the behavior of the animals outside their burrows indicates a reaction to gravity. If we suppose that a young animal entering the sand has sooner or later brought its axis approximately into coincidence with the direction of gravity, muscular elongation of the body will undoubtedly occur more frequently thereafter in this direction than in any other. This of itself is without doubt a factor in determining the shape of species without hard parts. After growth in the axial direction has begun and the body is more or less elongated, there is less resistance in the sand to growth in this direction than in any other. Moreover, the aboral end is not only an organ of attachment but a burrowing organ. So long as the end comes into contact only with the soft sand, the animal tends to extend and to force the end deeper and deeper. Thus these different factors combine to bring about greater growth in the longitudinal than in other directions. As was noted above (pp. 2–3), the lengths of the animals differ very greatly according to the depth of the layer of sand in which they live. The longest individuals I have ever found were in sand which was almost free from stones or broken shell for a depth of 40–50 cm. and many of the animals living there had attained this length and were attached. But these animals were not simply more elongated than those living under other conditions, they were actually larger. The diameter was fully as great as that of the largest specimens found under other conditions and in the contracted condition it was clearly evident that the individuals from the deep sand possessed much greater bulk than those from shallow sand. It is scarcely probable that the difference was due to difference in nutrition, for so far as it was possible to judge, the region of deep sand was much more barren of life and organic matter than the other. I think there can be no doubt that growth in the longitudinal direction is stimulated "functionally" so long as the aboral end does not come into contact with some solid surface to which it can attach.

In animals which possess no hard parts and in which muscles and supporting tissues form an important part of the body, the shape of the body, the direction of growth, and the relative size of various parts, *must* be determined, at least in large measure, by the functional conditions accompanying muscular contraction and by other mechanical factors. These factors may act either directly, as deforming factors, or indirectly through the "functional" stimulus, or they may act in both ways. Such species determine their own form in greater or less degree by their behavior. If we alter experimentally the character or energy of their reactions some more or less extensive morphological change is likely to occur. In such cases it is merely the capacity for reaction that is given in the germ, *i. e.*, inherited. The actual result in any given case depends on the internal and external conditions of development.

In the "Studies on Regulation" I have discussed the significance of certain mechanical and "functional" factors for the shape and certain other features of the structure of turbellaria, and in "Form Regulation in *Cerianthus*" for this form. The regulation of *Harenactis* in altered environment shows very clearly that similar factors are important in determining shape and other structural relations in this species, and the importance of these factors will appear still more clearly from the data of restitution.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
October, 1908.

BIBLIOGRAPHY.

Child, C. M.

'03 Form Regulation in Cerianthus. I. The Typical Course of Regeneration. Biol. Bull., Vol. V., No. 5, 1903.

'04a Form Regulation in Cerianthus. IV. The Rôle of Water-pressure in Regeneration. Biol. Bull., Vol. VI., No. 6, 1904.

'04b Form Regulation in Cerianthus. V. The Rôle of Water-pressure in Regeneration: Further Experiments. Biol. Bull., Vol. VII., No. 3, 1904.

'04c Form Regulation in Cerianthus. VI. Certain Special Cases of Regulation and their Relation to Internal Pressure. Biol. Bull., Vol. VII., No. 4, 1904.

'04d Form Regulation in Cerianthus. VII. Tentacle-Reduction and other Experiments. Biol. Bull., Vol. VII., No. 6, 1904.

'05 Form Regulation in Cerianthus. VIII. Supplementary Partial Discs and Heteromorphic Tentacles. Biol. Bull., Vol. VIII., No. 2, 1905.

Loeb, J.

'91 Untersuchungen über physiologischen Morphologie der Thiere. I. Ueber Heteromorphose. Würzburg, 1891.

Torrey, H. B.

'02 Papers from the Harriman Alaska Expedition. XXX. Anemones, with Discussion of Variation in Metridium. Proceedings of the Washington Academy of Sciences, Vol. IV., 1902.

ON THE USE OF CARBON DIOXIDE IN KILLING MARINE ANIMALS.

ALFRED G. MAYER.

For several years I have been making use of sea-water charged with CO_2 for producing anæsthesia in marine animals, thus enabling one to kill them in an expanded state.

One of the soda-water siphon-bottles, sold in the market under the trade name of "Sparklet Bottles," is filled with sea-water and this is charged with CO_2 by means of the "Sparklet bulb."

This charged sea-water is then poured into a vessel containing ordinary sea-water in which the marine animals are living, and in a few moments they are completely narcotized, and may then be killed in a fully expanded state by the addition of any killing fluid.

In some instances the CO_2 should be followed by the addition of a small quantity of chloretone, but this appears to be necessary only in the case of Siphonophoræ wherein it entirely prevents the casting off of the swimming bells.

This simple method enables one to obtain excellent preparations for histological or for museum purposes of such delicate marine animals as the medusæ. It is also applicable to Crustacea, Worms, Mollusca, etc.

Carnegie Institution Laboratory,
Tortugas, Florida.

EFFECTS OF REMOVING THE GERM-CELL DETERMINANTS FROM THE EGGS OF SOME CHRYSOMELID BEETLES. PRELIMINARY REPORT.[1]

R. W. HEGNER.

A number of experiments were made during the spring and summer of 1908 in order to test the conclusions reached in a previous study of the origin and early history of the germ-cells in *Calligrapha multipunctata* and several other chrysomelid beetles. These experiments suggested some interesting hypotheses which will be verified or disproved as soon as more material can be obtained. The work was begun at the Zoölogical Laboratory of the University of Wisconsin and continued at the Marine Biological Laboratory, Woods Hole, Mass.,[2] and at the Zoölogical Laboratory of the University of Michigan.

In a paper soon to be published[3] I have shown that there is an intimate connection between the primordial germ-cells and a disc of granules lying near the posterior end of the eggs of *Calligrapha multipunctata*, *C. bigsbyana*, *C. lunata* and *Leptinotarsa decemlineata*. This study led to an attempt to secure confirmatory experimental evidence. A brief account of the structure of the beetle's egg and the early history of the germ-cells is necessary for a clear understanding of the method of procedure and of the results.

The freshly laid eggs of these beetles consist of a thin peripheral layer of cytoplasm (the "Kleimhautblastem" of Weismann) and a relatively large central mass composed of yolk globules with a small amount of cytoplasm filling the interdeutoplasmic spaces. When the egg is deposited, the germinal vesicle, has already moved. to a point near the ventral surface just a trifle anterior to the center of the egg; here polar-body formation takes place. One or more male nuclei are present near the anterior end of the egg. Suspended in the "Keimhautblastem" near the posterior end of the egg is a mass of deeply staining granules which I have called the "pole-disc." These granules occupy the inner portion of the peripheral cytoplasmic layer and cover about one eighth of the area at this end of the egg (Fig. 1). Surrounding the egg are the vitelline membrane and the chorion.

---

[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 120.

[2] The writer is indebted to the Wistar Institute of Anatomy and Biology for the use of a room at the Marine Biological Laboratory, Woods Hole, Mass., during the summer of 1908.

[3] "The Origin and Early History of the Germ-Cells in some Chrysomelid Beetles." Accepted for publication by the *Journal of Morphology*.

After the conjugation of the sperm-nucleus with the egg-nucleus, which occurs a few hours after the eggs are laid, the cleavage nuclei increase rapidly in numbers and migrate in all directions toward the periphery reaching the "Kleimhautblastem" at the expiration of eighteen hours. Those nuclei (sixteen in

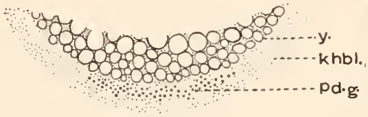


FIG. 1.

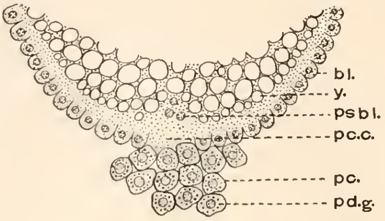


FIG. 2.

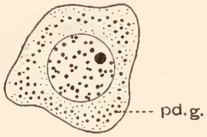


FIG. 3.

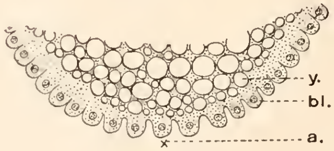


FIG. 4.

FIG. 1. A portion of the posterior end of an egg of *C. bigsbyana* in longitudinal section showing the arrangement of the pole-disc granules (*pd.g*). *y* = yolk; *khbl* = "Keimhautblastem."

FIG. 2. Longitudinal section through the posterior end of an egg of *C. bigsbyana* showing the primordial germ-cells (*pc*) containing pole-disc granules (*pd.g*). Nuclei which are prevented from forming blastoderm cells produce a pseudoblastodermic syncytium (*psbl*). The letters *pc.c* indicate the canal through which the pole-cells later migrate into the embryo. *bl* = blastoderm. *y* = yolk.

FIG. 3. A single primordial germ-cell showing pole-disc granules (*pd.g*).

FIG. 4. Longitudinal section through the posterior end of the egg of *C. lunata* described in Exp. 2*a*. The letter *a* indicates where germ-cells would be found in an uninjured egg. *bl* = blastoderm. *y* = yolk.

number) that come in contact with the granules of the pole-disc, do not remain in the "Kleimhautblastem" and later become the centers of blastoderm cells as do the other nuclei but become completely surrounded by a thin layer of these darkly staining bodies and continue their migration until they are entirely separated from the egg. They then lie between the vitelline membrane and the blastoderm in a closely packed group (Fig. 2). Each of these sixteen cells ("pole-cells") has the appearance of the one shown in Fig. 3. They soon divide by mitosis to form thirty-two. During this division the pole-disc granules are equally distributed among the daughter cells. Another division increases the number to sixty-four. This number is reached at the end of twenty-four hours and no further divisions take place until about the time of hatching. The blastoderm cells increase rapidly in number and can be distinguished from the cells just described not only because of the absence of granules but also by the smaller size of their nuclei. No difficulty was experienced in tracing the sixty-four cells containing pole-disc granules until they became the definite germ-gland.

These phenomena give reasonable grounds for the following conclusions. All the cleavage nuclei in the eggs of the above named beetles are potentially alike until in their migration toward the periphery they reach the "Keimhautblastem." Then those which chance to encounter the granules of the pole-disc are differentiated by their environment, *i. e.*, the granules, into germ-cells; all the other cleavage products become somatic cells. The granules of the pole-disc are therefore either the germ-cell determinants or the visible sign of the germ-cell determinants. The term determinant is not used here in the Weismannian sense but simply is meant to describe the material which fixes the character of the cells.

At first an attempt was made to tie off the posterior end of freshly laid eggs by means of fine silk thread in order to remove the pole-disc. This proved impossible for in every case the pressure burst the chorion. It was later found that if the chorion is punctured with a needle at any point a small drop of the contents is forced out by the turgidity of the egg and can be removed with filter paper. The eggs of these insects are perfectly oriented when laid. They were carefully placed with the posterior end up in small cavities in a block of paraffine. With the aid of a binocular microscope the point of a fine needle was then inserted at the center of this end, thus allowing that portion of the egg containing the pole-disc granules or primordial germ-cells to flow out. After removing this the eggs were placed in watch glasses and allowed to develop to the desired stage. Some difficulty was experienced in removing the proper amount of material and a number of the eggs either developed into shapeless masses of tissue or did not fix properly. These sources of error are in large part responsible for the failure to obtain more definite results.

I. The Effects of Removing the Pole-Disc Granules.

1. *C. multipunctata*, Exp. C.m. 1. — One egg of *C. multipunctata* was laid on April 15, 1908, and operated on while fresh. The portion removed from the posterior end was fixed on a slide and stained. It was found to contain the pole-disc granules. This egg did not hatch in the average period (5⅔ days) and so was preserved at the age of 8 days.[1] The embryo appeared to be normal in every way, but on sectioning it was found to be lacking not only in germ-cells but also in other abdominal tissues. The mid- and hind-intestines were not completely formed and other neighboring parts were likewise deficient. It is evident that the germ-cell determinants were removed by the operation and that so much of the other organ-forming substances was also removed that the embryo failed to develop the corresponding tissues.

2. *C. lunata*, Exp. C. 1. — An egg of *C. lunata* which was laid at 12 o'clock noon July 16, was operated at 2 p. m. on the same day and fixed forty-nine hours later. It was stained in toto with hæmalum, drawn and then sectioned. Externally it resembled exactly embryos of this age developed from entire eggs. Germ-cells were found in the last two abdominal segments in a position similar to that occupied by them in normal embryos. However, there were not sixty-four, as was found to be the case in embryos of *C. multipunctata*, but only thirty germ-cells could be

---

[1] It has often been observed that when eggs of this beetle do not hatch the embryos live for several days after the expiration of the regular hatching period.

found. It seems probable that not all of the pole-disc granules were removed in the operation and that those which were left behind determined the character of the cells which encountered them, the result being a smaller number of germ-cells.

3. *C. lunata*, Exp. C. 3. — This larva hatched at noon on July 22, exactly six days after the egg was laid. This constitutes a period eight hours longer than has been computed as the average time for eggs of this species.[1] Apparently very little if any delay in development was caused by the operation. This larva was normal in external appearance and was as active as those hatched from eggs which had not been injured. It was killed when three days old. No germ-glands could be discovered on sectioning, but as several sections were ruined by an accident no incontestible statement as to their presence or absence can be made.

4. *C. lunata*, Exp. 2*a*. — This egg was laid and operated on at 10 a. m. July 24 and was fixed at the expiration of twenty-two hours. At this age a normal egg has a group of primordial germ-cells between the vitelline membrane and the blastoderm at the posterior end (Fig. 2). No germ-cells are present in the operated egg; the blastoderm cells at the posterior pole (Fig. 4) are less numerous than at other points on the surface indicating a delayed development in this region due to the removal of the "Keimhautblastem."

5. *C. lunata*, Exp. 2*b*. — This egg was laid and operated on at the same time as the egg in Exp. 2*a*. It was preserved when thirty-six hours old. At this stage in uninjured eggs the germ-cells are present as a group near the posterior end of the ventral groove. No germ-cells were found in the operated egg but a mass of yolk and cytoplasm was discovered outside of the vitelline membrane near the posterior end.

II. The Effects of Removing the Primordial Germ-Cells.

In two experiments on stages shown in Fig. 2 eggs were punctured and the germ-cells allowed to flow out. The results indicate that all of the germ-cells were not removed in every case.

---

[1] For an account of the life histories of these beetles see R. W. Hegner, "Observations on the Breeding Habits of three Chrysomelid Beetles, *Calligrapha bigsbyana*, *C. multipunctata* and *C. lunata*," *Psyche*, Vol. 15, pp. 21–24.

It is the intention of the writer to repeat these operations next spring.

1. *C. lunata*, Exp. 6*b*. — An egg which was laid at 11 a. m. on July 23, was allowed to develop for twenty-five hours and then was operated on. It was preserved forty-four hours after the operation. Twenty-three germ-cells instead of sixty-four were found occupying the normal position in the embryo. Four of these twenty-three cells were in the posterior amniotic cavity while nineteen were scattered among the mesoderm cells in the last two abdominal segments. This difference in number can be accounted for by supposing that part of them were removed in the operation.

2. *C. lunata*, Exp. 6*c*. — A larva hatched from an egg which was laid and operated on at the same time as the egg in Exp. 6*a* The larva was preserved at once. No germ-glands could be found in the sections. Several sections broke on the knife but these were from a part of the larva outside of the germ-gland region.

3. *L. decemlineata*, Exps. p. 1–7. — It is evident from the results of these experiments that either all of the primordial germ-cells were not removed from the egg during the operation or else some other cells have taken on the function of germ-cells. In one case (*L. decemlineata*, Exp. *p.* 2) it is certain that all the pole-cells were not removed. In two instances (Exps. *p.* 4 and *p.* 6) germ-glands were undoubtedly present. The method of operating therefore needs to be improved before decisive results can be secured.

III. THE EFFECTS OF REMOVING OTHER PORTIONS OF THE EGG.

Only one experiment was made to note the effect of removing any part of the egg other than the posterior end. The anterior end of a freshly laid egg of *C. bigsbyana* (Exp. *C. b.* 1) was punctured and a large drop of the contents removed. A normal larva hatched from this egg, passed through the larval and pupal stages and is now hibernating as an adult.

The above experiments prove that a part of an insect's egg may be removed without preventing the development of the embryo and subsequent hatching of the larva. More work must be

TABLE GIVING THE NATURE AND RESULTS OF ALL THE EXPERIMENTS.

Name.	No. of Experiment.	Age of Egg When Operated.	Operation.	Interval Between Operation and Fixation.	External Condition of Embryo or Larva.	Sections.	Germ-Cells.	Histological Condition.
C. multipunctata	C. m. 1	fresh	pole-disc removed	8 days	normal	perfect	not present	imperfect
C. lunata	C. 1	2 hours	"	49 hours	"	"	fewer than normal	perfect
"	C. 2	"	"	97 "	"	"	not found	"
"	C. 3	"	"	9 days larva 3 days	"	several lost	"	"
"	2. a	fresh	"	22 hours	post. end altered	perfect	"	"
"	2. b	"	"	36 "	"	"	"	"
"	2. c	"	"	48 "	abnormal	"	"	abnormal
"	2. d	"	"	72 "	ruined by accident			
"	2. e	"	"	7 days	abnormal	perfect	not found	abnormal
"	5. a	"	"	24 hours	ruined by accident			
"	5. b	"	"	44 "	"			
"	5. c	"	"	68 "	abnormal	perfect	not found	abnormal
"	5. d	"	"	9 days	"	"	"	"
L. decemlineata	L. d. 1	"	"	10 " 4 day larva	normal	"	"	normal
"	L. d. 2	"	"	72 hours	"	several lost	"	"
"	L. d. 3	"	"	6 days 1 day larva	"	"	"	"
"	L. d. j	"	"	48 hours	"	perfect	"	"
C. lunata	6. a	25 hours	primordial germ-cells removed	24 "	"	"	"	"
"	6. b	"	"	44 "	"	"	fewer than normal	"
"	6. c	"	"	7 days 1 day larva	"	several lost	not found	"
L. decemlineata	p. 1	control	none		"	perfect	present	"
"	p. 2	26 hours	primordial germ-cells removed	fixed at once	"	"	fewer than normal	"
"	p. 3	"	"	16 hours	abnormal	"	not found	abnormal
"	p. 4	"	"	48 "	normal	"	present	normal
"	p. 5	"	"	72 "	abnormal	several lost	not found	abnormal
"	p. 6	"	"	104 " 1 day larva	normal	perfect	present	normal
"	p. 7	"	"	8 days 4 day larva	"	several lost	not found	"
C. bigsbyana	C. b. 1	fresh	ant. end removed		hibernating (Oct. 30, 1908) as an adult			

done before any final statement can be made as to the effects of removing the germ-cell determinants (pole-disc granules), but it seems probable that if all of these are taken from the egg no germ-cells will be produced. Previous researches have proven beyond a doubt that the pole-disc granules are taken up by the primordial germ-cells. It remains however for future experiments to decide whether these granules are really the germ-cell determinants or are non-essential in the differentiation of the cleavage products.

Zoölogical Laboratory,
University of Michigan,
October 30, 1908.

Vol. XVI. *January, 1909.* *No. 2*

BIOLOGICAL BULLETIN

STUDIES ON SEX-DETERMINATION IN AMPHIBIANS. II.

HELEN DEAN KING.

It is still an open question whether external factors have any influence on the determination of sex. Much of the experimental work that has seemed to show that sex can be influenced by nutrition, temperature, etc., has either been carried out with too small a number of individuals or the methods used in the experiments have been too crude to give results that could be considered as decisive. It is seemingly quite within the power of the modern investigator to determine definitely whether sex can be influenced by external factors; and careful work, such as that which has been done by Cuénot and Hertwig, should settle this question for all time. If it is found that external factors do influence sex then the current chromosome-sex theory will have to be abandoned or considerably modified; if, on the other hand, it is shown convincingly that external factors have no influence on sex-determination, then the way is cleared for the theory that best explains the facts known to us and offers the most favorable hypothesis for future investigations along this line.

Several years ago I started a series of experiments on the eggs of the common American toad, *Bufo lentiginosus*, in order to study the problem of sex-determination in this amphibian which seems to furnish peculiarly favorable material for an investigation of this kind. In the first experiments that were made the influence of nutrition on the determination of sex was investigated. The results of this work, as shown in a previous paper (King, '07), seem to indicate that neither the quantity nor the quality of the food given the larvæ has any influence on sex. The present paper records the results of the second series of experiments that was made to study the determination of sex in *Bufo*. The work was carried on in the vivarium of the University of Pennsylvania where the splendid facilities at my command permitted the use of relatively large numbers of individuals in each experiment and the rearing of them under exceptionally favorable conditions.

In all of the experiments in this series the tadpoles were kept in large glass aquaria or in cement tanks which were supplied with running water. Each tank contained a sloping bank of sand and gravel and also a quantity of water plants. With the exception of the one experiment in which the influence of starvation on the determination of sex was being studied, all of the tadpoles used in these investigations received similar food, viz., water plants and minute organisms in abundance and occasionally fine pieces of cooked meat or cereal. According to my observations tadpoles of *Bufo* invariably prefer an animal to a vegetable diet and they readily devour the dead bodies of their companions. To this latter fact can be attributed the loss of many individuals during the course of these experiments. Since there is no apparent relation between mortality and sex among tadpoles reared under artificial conditions, as shown by the investigations of Pflüger ('82), and also by my former work, the individuals in which sex was not ascertained are disregarded in considering the results of these experiments, although the total number of individuals with which each experiment started is given in most cases. The methods used in ascertaining the sex of the individuals were similar to those previously employed (King, '07). Whenever possible the bodies of tadpoles that died were preserved and the sex ascertained by means of sections. As there is considerable individual variation in the size and also in the development of the gonads, even after the metamorphosis of the toads, it was necessary to section the genital organs of nearly one fourth of the total number of individuals in order to ascertain their sex.

I. Experiments to Determine the Sex Ratio of the Individuals that Develop from the Eggs of the Right Ovary and of those from the Left Ovary.

If sex is determined in the ovary, as many investigators believe, it is possible that the individuals developing from the eggs of one ovary may show a very different sex ratio from that of the individuals produced from the eggs of the other ovary ; or it may be that the eggs of one ovary produce only females and those of the other ovary only males. The following experiments were made to determine this point for *Bufo*. On April 26, 1907, a female which had just begun to deposit her eggs was killed by pithing. The eggs in the right uterus were separated from those in the left uterus and each set was artifically fertilized, sperm from the same male being used for both lots. Six hundred eggs from each ovary were taken for the purposes of the experiment and placed in tanks of equal size. During the course of their development all of the tadpoles were subjected to similar conditions of temperature and they received similar food. The sex of the first 300 individuals in each set to undergo metamorphosis was ascertained with the result shown in the following table :

Table I.

	Total Sex Ascertained.	Males.	Females.	Per Cent. of Females.
Right Ovary................	300	131	169	56.33
Left Ovary..................	300	128	172	57.33
Total......................	600	259	341	56.83

As shown in the above summary both lots of individuals gave approximately the same sex ratio ; 56.33 per cent. of the individuals from the right ovary and 57.33 per cent. of those from the left ovary being females. This proportion of females is somewhat higher than that obtained in the majority of the experiments and seems to indicate that there is considerable normal variation in the sex ratio of lots of eggs laid by different females.

The above experiment was repeated with the eggs of another toad in the spring of 1908. Owing to an accident, however, all of the tadpoles developing from the eggs of the left ovary were killed and in only 140 of the individuals that had developed from the eggs of the right ovary was sex ascertained. Of this number 64 individuals were males and 76, or 54.2 per cent., were females.

From the results obtained in these two experiments it is evident that the sex ratio of the individuals produced from the eggs of the right ovary is not materially different from that of the individuals which come from the eggs of the left ovary. Whatever the factors may be which normally determine sex in *Bufo* they appear to have a similar action on the eggs of both ovaries.

II. The Influence of Starvation on Sex-Determination in Bufo.

During the course of my former experiments an attempt was made to ascertain whether a scarcity of food tends to produce a relatively greater number of males, as several investigators have maintained. The mortality among the young tadpoles was so great, however, that the experiment was abandoned for the time. In the spring of 1907 this experiment was repeated on a much larger scale. Eight hundred eggs which had been laid and normally fertilized in the laboratory on the morning of March 30, were placed in a large tank containing only water and clean sand. No food of any kind was given the tadpoles at first and, as might be expected, they began dying in great numbers about two weeks after hatching. Until the beginning of May the only food that the larvæ obtained was the dead bodies of their companions which were devoured before they could be removed. The tadpoles that lived developed very slowly, and in order to prevent all of them from dying they were given a small amount of meat on the second of May and once a week thereafter. Of the 800 individuals with which the experiment started only 59 lived until their sex could be ascertained. Of this number 24 were males and 35, or 59.32 per cent., were females. This number of individuals is, of course, too small to be of much value for statistical purposes, yet the results of the experiment, as far as they go, seem to indicate that scarcity of food has no influence whatever on sex-determination in *Bufo*. Since the only food that the tadpoles received was animal food it might be inferred, from the rather large proportion of females that were obtained, that nitrogenous food favors the production of females, as Yung ('85) has maintained. The excess of females does not seem to me sufficiently large, however, to warrant such a conclusion. Moreover my previous experiments showed that in a total of 464 individuals that received an exclusive meat diet only 246, or 53.01 per cent., were females, thus making it highly improbable that nitrogenous food has any influence on the determination of sex in *Bufo*.

III. The Influence of the Ripeness of the Egg at the Time of Fertilization on Sex-Determination in Bufo.

For several years past Hertwig ('05–'07) has been investigating the problem of sex-determination, principally in various species of *Rana*, and he has already published three contributions to the literature on this subject. In his first paper, which appeared in 1905, Hertwig ('05) suggests that the sexual character of an egg changes during the course of its development in the ovary. In the early stages of its ripening the egg tends to produce a male ; in the middle phase of its ripening its tendency is towards the production of a female ; and in later phases, when the egg is overripe, it again shows a tendency to develop into a male. This change in the sexual character of the egg Hertwig ascribes in his second paper ('06) to "einem verschiedenen Wechselverhältnis ihrer Hauptbestandteile, der Kernsubstanz und des Protoplasma. . . . Eier, welche relativ ärmer an Kernsubstanz sind, Weibchen liefern, chromatinreichere dagegen Männchen." In his latest communication Hertwig ('07) lays little emphasis on the second part of his theory, but he seems to consider that the results of his experiments warrant the conclusion that the ripeness of an egg at the moment of its fertilization determines its sex. In explaining the results of a certain set of experiments in which the eggs of a female from one locality were fertilized with sperm from a male taken from a different locality, Hertwig further modifies his theory by the suggestion that in some cases the spermatozoön exerts a definite influence on sex-determination, although, as a rule, it is probable that "die Eier zur Zeit der Befruchtung sexuell in so hohem Grad determiniert sind, das der relativ geringe Einfluss des Samens gar nicht zur Geltung kommen würde."

Morgan ('08) has recently given a brief review of this work of Hertwig's on sex-determination and also a criticism of the results. He states that "Hertwig's attempt to connect his view with the ratio of nucleus to cell-plasm of the egg at different periods of its maturation can hardly be looked upon favorably, since in the frog's egg the nucleus as such has already disappeared when the egg leaves the ovary. The chromosomes are thereafter arranged on the equatorial plate of the first polar spindle. It is, however, during this period that the degree of ripening is supposed to determine the sex of the egg." Morgan suggests that the results of Hertwig's later experiments seem to show that the male is responsible for sex-determination. The preponderance of one sex over the other would be explicable on the assumption that "more sperm of one kind, if two kinds exist, are injured or that internal processes may lead to the production of more functional sperm of one sex." It is unfortunate for this suggestion of Morgan's that the numerous investigations which have been made of the spermatogenesis of various species of amphibians have so far failed to show the slightest evidence of a dimorphism in the spermatozoa.

The frog offers much more favorable material than the toad for an investigation of the influence of the ripeness of the egg at the time of fertilization on sex-determination, since it is possible to extend the laying period of a frog over several days while the toad lays all of her eggs within a few hours whether she is separated from the male or not. Two different experiments were made with the eggs of *Bufo* in order to study this problem. In the first experiment a pair of toads were used that had been brought into the laboratory early in the morning of March 30, 1907. The female commenced laying at 10.45 A. M. of the same day. At 11.15 A. M. the toads were disturbed and the eggs already laid (Lot *A*) were removed to a jar of fresh water. The female began laying again at 12.05 P. M and a second set of eggs (Lot *B*) were removed at 12.25 P. M. The third laying (Lot *C*) began about 1 P. M. and was interrupted at 1.15 P. M.; while the fourth and last lot of eggs (Lot *D*) were deposited between 2.15 P. M. and 3 P. M. The laying period for this female, therefore, extended over about four hours. Four hundred eggs were taken from each lot for the purposes of the experiment, each set of eggs being put in a compartment of a large aquarium. The compartments were all approximately of the same size and each contained about the same amount of water and of plant food. All of the 1,600 individuals used in this experiment were, therefore, subjected to similar external conditions during the period of their development. There was no appreciable difference in the rate of growth of the tadpoles in the different compartments and the mortality was nearly the same in each lot. The results of this experiment are summarized in the following table.

TABLE II.

	Total Number Individuals.	Total Sex Ascertained.	Males.	Females.	Per Cent. of Females.
Lot *A*..........	400	149	70	79	53.02
Lot *B*..........	400	202	88	114	56.43
Lot *C*..........	400	133	56	77	57.89
Lot *D*.........	400	167	78	89	53.29
Total.........	1,600	651	292	359	55.14

The results of this experiment show a striking uniformity since in all four lots about the same proportion of the individuals in which sex was ascertained were females. The excess of females was slightly greater in the two middle lots (*B* and *C*) than in Lots *A* and *D*. This result would seem to support Hertwig's contention that eggs in the middle phase of ripening at the time of fertilization tend to produce a greater proportion of females. The extreme lots of the series (*A* and *D*) did not, however, give a correspondingly greater proportion of males as the theory demands. It is very probable, therefore, that the slightly greater proportion of females in the two middle lots has no especial significance. The comparatively short interval between the laying of the eggs in Lot *A* and of those in Lot *D* makes no direct comparison possible between the results of this experiment and those of Hertwig on *Rana*, since in some of Hertwig's experiments there was an interval of two or three days between the laying of the different lots of eggs by the same female. Normally the eggs of the frog, as well as those of the toad, are all laid within a short period of time. It would seem, therefore, as if in the above experiment a relatively greater number of males should have developed from the eggs of Lots *A* and *D* if, as Hertwig believes, the ripeness of an egg at the time of fertilization has any influence on its sex and if eggs fertilized in early or in late phases of ripening have a tendency towards male production.

All of the eggs used in this experiment should have been peculiarly favorable for the production of males according to Hertwig's theory, since, owing to the very early spring in 1907, the eggs were laid fully one week earlier than is the usual breeding time for toads in eastern Pennsylvania. Of the 651 individuals in which sex was ascertained 359, or 55.14 per cent., were females. This result does not accord with Hertwig's view that the younger the egg at the time of its fertilization the greater its tendency towards male production. No eggs were obtained in the spring of 1907 from the end of March until the last week in April when several pairs of toads were brought into the laboratory and a large number of eggs laid and normally fertilized. According to Hertwig's theory these eggs were all overripe and should, therefore, have given a preponderance of males. To this series belong the eggs used in the experiment described in section 1. The results of the experiment in question, which are summarized in Table I., show that in the total of 600 individuals in which sex was ascertained there were 341 or 56.83 per cent. of females. This is about the proportion of females that is apparently normal for the species under natural conditions, and this normal sex ratio does not seem to be altered whether the eggs are laid the latter part of March or retained by the female until the end of April.

The second experiment that was made to test the influence of the ripeness of the egg at the time of fertilization on sex-determination was carried out as follows: A female which was isolated on the afternoon of April 5, 1908, began laying at 2 A. M. the following morning. She was at once killed by pithing and the body, unopened, placed in a moist chamber. At 9 A. M., seven hours later, the eggs were removed from the body of the female and artificially fertilized, sperm from two males being used. Although the eggs appeared perfectly good many of them did not fertilize, and the segmentation of the fertilized eggs was slow and in many cases abnormal. It is evident, from this fact, that post-mortem changes had already begun in the eggs at the time that they were fertilized and that it would not be possible to obtain normal embryos from eggs of *Bufo* that remained unfertilized much longer than seven hours after the death of the female. Although the eggs in this series were placed under the most favorable conditions possible many of them failed to gastrulate and the tadpoles that lived developed very slowly, large numbers of them dying in the early stages of their development. Of the thousand or more eggs with which the experiment started only 372 tadpoles lived until it was possible to ascertain their sex, and in fully one half of this number the gonads had to be sectioned in order to make sure of the sex. Of the 372 individuals in which sex was ascertained 178 were males and 194, or 52.15 per cent., were females. The sex ratio found in these individuals which developed from "overripe" eggs agrees essentially with that found among the individuals in the first experiment where presumably the eggs were in very early phases of ripening at the time that they were fertilized. In both of these experiments an excess of females was produced, although, to accord with Hertwig's theory, there should have been an excess of males in each case.

The results of these experiments seem to indicate that in *Bufo* sex is not determined by the ripeness of the egg at the time that it is fertilized. Eggs fertilized normally within a period of four hours or artificially seven hours after the death of the female, eggs laid the last of March as well as those laid near the end of April, all produce a slight excess of females and thus give a sex ratio that is practically the same as that found among young toads that have recently completed their metamorphosis under natural conditions.

In connection with these experiments reference may be made to a theory of sex-determination recently elaborated by a physician, Dr. T. E. Reed ('07). Starting with the supposition that the ovum is hermaphroditic and that sex, which is a property or function of the ovum, is determined at the time of the fertilization of the egg, Reed then assumes that there is a sex cycle in the germ-plasm and that "this rhythm extends over a period of twelve hours, six being active or masculine, and six passive or feminine, and changes from day to day as do the tides. . . . The sex of the embryo is determined simply by the period through which the germ plasm happens to be passing when fertilization takes place." According to this theory if an egg is fertilized during the positive period, *i. e.*, when the tide is rising, the resultant embryo is always a male; if fertilization takes place during the negative period, when the tide is ebbing, the embryo is necessarily a female. Reed gives no statistics regarding the working out of this hypothesis, but he states that it holds good for a score or more of cases in man and a number of cases in the breeding of horses and cattle. He does not explain, however, how the theory works in the case of such animals as dogs, pigs, rats, etc., which produce a number of offspring of both sexes at one birth. It can hardly be supposed that the fertilization of the ova in all of these animals invariably takes place "at the turn of the tide" when, according to this theory it might be possible for some of the ova to be fertilized during the positive (male) period and others during the negative (female) period.

An opportunity was offered to test this theory for *Bufo* with the lot of eggs used for the first experiment described in this section. On March 30, 1907, the tide was high at Philadelphia, where the experiments were being carried on, at 2.22 P. M. According to Reed's theory the six hours previous to this time woud form the positive period during which all eggs fertilized should develop into males. Of the eggs used for the experiment in question Lots *A*, *B* and *C* were laid and fertilized within the positive period and should, therefore, all have become males. Lot *D*, which was laid about the time of the turning of the tide, might be supposed to produce some females. This last lot, as a matter of fact, did not produce as great a proportion of females as did Lot *B*, which was laid at the middle of the positive period and therefore, according to Reed, at the most favorable time for the production of males. Results similar to these were obtained with another lot of eggs laid within the positive period. Reed's hypothesis, therefore, is not adequate to explain the determination of sex in *Bufo* however valuable it may prove to be as a theory of sex-determination in some of the higher mammals.

IV. The Influence of Temperature on the Determination of Sex in Bufo.

It has been held by several investigators that temperature has a decided influence on sex-determination ; a high temperature favoring the development of females, a low temperature tending towards male production. Most of the experiments which have seemed to give support to this hypothesis have been carried out with the lower forms, such as *Daphnia* and *Hydatina*. The only experiments which have seemed to show that temperature has an influence on sex-determination in the vertebrates are those recently made by Hertwig on *Rana*.

In the first experiment which he made to study the influence of temperature on sex-determination Hertwig divided a bunch of eggs of *Rana temporaria* into two lots; one lot of eggs was allowed to develop at a temperature of 25° C., the other lot developed at a temperature of 13° C. The mortality among the tadpoles that were subjected to the higher temperature was exceedingly great. Out of a total of 200 individuals only 67 underwent metamorphosis, and of this number 4 were males and 63 females. The individuals placed in the cold water lived much better that the others, although the development of their genital organs was so greatly retarded that their sex could not be ascertained even at the time of metamorphosis. In a second series of experiments on the eggs of *Rana esculenta* Hertwig obtained the result shown in the following table.

TABLE III.

Water Temperature.	Males.	Females.	Per Cent. of Females.
22–30° C.	245	127	34.13
13–21° C.	282	54	16.07

Hertwig considers that the results of these two sets of experiments indicate that a high temperature favors the development of females, although he states that a number of individuals in which sex was ascertained is too small to give a decisive answer to the question. In the second experiment the females formed but 34.13 per cent. of the individuals that had developed in the warm water and but 16.07 per cent. of those that had been reared in the cold water. As this experiment stands it hardly seems to me to offer any evidence in support of the view that a high temperature favors the development of females. Both lots of eggs gave a large proportion of males and normally, according to the investigations of von Griesheim ('81) and of Pflüger, there appears to be an excess of females among both young and adult frogs. It would be interesting to know under what conditions the eggs used in this second experiment were laid as this knowledge might help to explain the reason for the extraordinary excess of males.

Hertwig repeated this experiment in 1906 using eggs of *Rana temporaria.* None of the tadpoles developing at a temperature of 20° C. lived to undergo metamorphosis and therefore their sex could not be ascertained. Out of 340 larvæ developing at a temperature of 10° C., 74 lived to undergo metamorphosis. Of this number 38 were males, 23 females, and in 11 the sex was not ascertained although the individuals were probably males. Hertwig's conclusion from this experiment is that cold favors the development of males as heat favors the development of females.

The following series of experiments was made to study the influence of temperature on the determination of sex in *Bufo.* Five hundred eggs laid and normally fertilized in the laboratory on the morning of March 31, 1907, were divided into two lots: one lot of 250 eggs was placed in a greenhouse, heated by steam, where the temperature of the water in which the tadpoles were developing varied from 23°–30° C.; the remaining 250 eggs were placed in a tank in an unheated basement room where the temperature of the water in which the tadpoles lived ranged from 14°–18° C. during the daytime and was probably much colder at night. Both lots of tadpoles were given similar food and all of the conditions, excepting the temperature, were made as nearly alike as possible. For convenience in description this set of 500 eggs will be called Lot *A.* An unusually high temperature has apparently as injurious an effect on the eggs of *Bufo* as it has on the eggs of *Rana* since a great many of the tadpoles that were kept in water with a temperature of 23°–30° C. died before it was possible to ascertain their sex. The tadpoles that lived developed very rapidly and many of them had completed their metamorphosis by the first of June. The tadpoles kept in the cold water were vigorous and healthy, and comparatively few of them died. Their development was very slow, however, as compared with that of the individuals living in the warm water. At the end of May the largest of the tadpoles in this lot had a body length of but 8 mm. and their hind legs were still very small. None of these tadpoles underwent metamorphosis until the third week in June.

From a second lot of eggs laid in the laboratory on March 31, 1907, another 500 eggs (Lot *B*) were taken and divided into two sets of 250 eggs each as in the previous experiment. These eggs were placed under conditions similar to those of Lot *A*. The tadpoles in this series behaved in all respects like those in the first series as regards their rate of development and time of metamorphosis. The following table summarizes the results obtained with these two lots of eggs.

TABLE IV.

Water Temperature.		Number Sex Ascertained.	Males.	Females.	Per Cent. of Females
Lot *A*	22–30° C.	63	19	44	69.84
Lot *B*		39	30	9	23.07
Lot *A*	14–18° C.	169	68	101	61.58
Lot *B*		117	73	44	37.60

As shown in the above table the sex ratio of the individuals used in these experiments differs considerably from the sex ratio which is presumably normal for the species. The individuals of Lot *A*, whether they had lived in warm water or in cold water, produced an unusually large proportion of females; in the one case 69.84 per cent., in the other 61.58 per cent. of the individuals in which sex was ascertained were females. The most striking deviation from the apparent normal sex ratio is shown in the individuals of Lot *B*. Here the number of females is only 23.07 per cent. in the case of the tadpoles that had been reared in the warm water and 37.60 per cent. among the individuals that were subjected to a temperature of 14°–18° C. during the course of their development. The results of these experiments fail to support Hertwig's contention that a high temperature acting during the course of the development of the tadpoles favors the production of females, while a low temperature tends to produce a relatively greater proportion of males. To be sure one set of eggs (Lot *A*) developing at high temperature gave 69.84 per cent. of females which is a considerably larger proportion of females than is found among young toads under natural conditions; but another set of eggs (Lot *B*) developing under exactly similar conditions produced but 23.07 per cent. of females. Just as striking a difference is shown in the case of the individuals that were reared in cold water. Lot *A* produced 61.58 per cent. of females, while Lot *B*, developing under similar conditions, gave but 37.60 per cent. of females. The very different sex ratios obtained with lots of eggs subjected to the same temperature conditions seem to indicate that sex in *Bufo* is not determined by the temperature of the water in which the tadpoles develop.

If sex is already determined in the egg and not influenced by external factors there is probably a considerable variation in the sex ratios of lots of eggs laid by different females. It might, therefore, be possible to explain the above results on the assumption that, by chance, batches of eggs were taken for the experiment which gave the extremes of possible normal variations in the sex ratio. This explanation is hardly a satisfactory one, especially in light of the results obtained in the experiment about to be described. The batch of eggs from which the individuals used in Lot *A* were taken was laid in water which ranged from 16°–18° C. during the two hours or more in which the eggs were being deposited and fertilized. The eggs used in Lot *B*, on the other hand, were laid in water which had a temperature of from 11°–13° C. during the period of their deposition. The results of the experiment at once suggested the idea that the temperature of the water at the time that the eggs were fertilized might possibly have some influence on sex; a high temperature favoring the development of females, a low temperature that of males. The following experiment was made to determine the value of this suggestion.

On April 5, 1908, a pair of toads were placed in water which remained at a temperature of about 26° C. during the time that the eggs were being deposited and fertilized. All of the eggs that were laid fertilized readily at this temperature and all segmented in a normal manner. For convenience in description this set of eggs will be called Lot *C*. On the same date another pair of toads were placed in water which had a temperature of about 9° C. during the period in which the eggs were being deposited. All of the eggs in this set, which will be called Lot *D*, were also fertilized and all developed normally. There was, therefore, a difference of about 17° C. in the temperature of the water in which these two sets of eggs were laid. The eggs of each lot, several thousand in number, were put in a large cement tank supplied with running water and containing plenty of food. The individuals in Lot *C* developed somewhat more rapidly than those in Lot *D* and they began their metamorphosis sooner. The experiment had to be discontinued on July 7, although a number of individuals in each set had not yet reached a stage of development when it would be possible to ascertain their sex. The results of the experiment, tabulated according to the weeks when the individuals died or completed their metamorphosis, is given in the following summary.

TABLE V.

	Lot *C*.			Lot *D*.		
Date.	No. Sex Ascertained.	Males.	Females.	No. Sex Ascertained.	Males.	Females.
May 24–June 6........	126	66	60	34	18	16
June 7–13...	328	160	168	132	98	34
June 15–20...	790	344	446	808	524	284
June 22–27 .	697	255	442	924	422	502
June 28–July 7........	237	128	109	185	119	66
Total...	2,178	953	1,225	2,083	1,181	902

As shown in the above table the sex was ascertained of 2,178 of the individuals that developed from the eggs laid in warm water. Of this number 1,225, or 56.24 per cent., were females, thus giving a sex ratio for Lot *C* that is little, if any, higher than that which is apparently normal for the species. The results of this part of the experiment, therefore, do not seem to favor the suggestion that a high temperature acting on the eggs at the time of their fertilization tends to produce a relatively greater number of females. There is the possibility, however, that this negative result may be due to the fact that in making the experiment a higher temperature was used than that which is the optimum for the production of the greatest number of females and that many more females would have developed had the eggs been fertilized in water with a temperature but slightly higher than that used in the former experiment with Lot *A*.

The sex ratio found among the individuals in Lot *D*, agreeing with that of Lot *B*, shows a considerable deviation from the sex ratio that is presumably normal for the species. In a total of 2,083 individuals which developed from eggs laid in water with a temperature of 9° C. there were 902 or 43.30 per cent. of females. Only once in the course of my investigations on sex-determination in *Bufo* have I found the proportion of females in a lot of individuals anywhere near as low as that found in Lots *B* and *D* in the above experiments. In studying the influence of nutrition on sex-determination in *Bufo* I found that in a total of 349 individuals that had been fed on mixed animal and vegetable food only 45.84 per cent. were females. Other lots of eggs laid by the same females gave as high as 59 per cent. of females however ; and on summarizing the results obtained in the entire series of experiments it was found that the females formed 53.58 per cent. of the total number of individuals in which sex was ascertained. In the last experiment described there were 2,127 or 49.91 per cent. of females among the 4,261 individuals whose sex was ascertained. This fact strongly suggests that the sex ratio in this lot of individuals was affected by the conditions under which the eggs were laid.

The results of the experiments described in this section indicate, although they by no means prove, that the temperature of the water in which the eggs are fertilized has some influence on the determination of sex in *Bufo.* A high temperature appears to favor the development of females ; a low temperature, on the other hand, seems to lead to the development of relatively more males. It is possible, as before suggested, that the results obtained in these experiments may be due to the fact that the sex ratio in *Bufo* is a variable one, the proportion of females ranging from 35–70 per cent. in lots of eggs laid by different females. If such is the case it surely is a strange coincidence that lots of eggs should have been taken for these temperature experiments which would give the greatest extremes in the sex ratio.

One serious objection that can be made to these experiments is that the eggs which were fertilized in warm water were laid by one female while the eggs fertilized in the cold water were laid by another individual. This gives an opportunity for the results to be influenced by possible normal variations in the sex ratio in different lots of eggs. The two sets of eggs were fertilized by sperm from different males also and there is the possibility, as Morgan has suggested, that the male is responsible for sex-determination in amphibians. The last experiment was carried out in the manner described in order to obtain as large a number as possible of eggs that were fertilized normally at different temperatures, since it is not safe to draw conclusions from the results of experiments in which comparatively few individuals are used. Another series of experiments will be made next spring which, it is hoped, will determine definitely whether temperature can influence sex in *Bufo*. An account of the experiments already carried out is published in the hope that other investigators working in the same field may consider the suggestion here made as of sufficient value to warrant testing on any suitable material with which they may be working.

LITERATURE.

von Griesheim, A.

'81 Ueber die Zahlenverhältnisse der Geschlechter bei Rana fusca. Arch. für die gesammte Phys., Bd. XXVI., 1881.

Hertwig, R.

'05 Über das Problem der sexuellen Differenzierung. Verhandl. der Deutsch. Zool. Gesellschaft, 1905.

'06 Weitere Untersuchungen über das Sexualitätsproblem. Verhandl. der Deutsch. Zool. Gesellschaft, 1906.

'07 Untersuchungen über das Sexualitätsproblem, III. Verhandl. der Deutsch. Zool. Gesellschaft, 1907.

King, Helen Dean

'07 Food as a Factor in the Determination of Sex in Amphibians. Biol. Bulletin, Vol. XIII., 1907.

Morgan, T. H.

'08 The Determination of Sex in Frogs. Amer. Naturalist, Vol. XLII., 1908.

Pflüger, E.

'82 Ueber die das Geschlecht bestimmenden Ursachen und die Geschlechtsverhältnisse der Frösche. Arch. für die gesammte Phys., Bd. XXIX., 1882.

Reed, T. E.

'07 The Sex Cycle of the Germ Plasm. New York Medical Times, 1907.

Yung, E.

'85 De l'influence des Variations du milieu physio-chimique sur le développement des animaux. Arch. des Sci. phys. et natur., t. XIV., 1885.

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY,
PHILADELPHIA, PA.

THE INFLUENCE OF SOME AMINO-ACIDS ON THE DEVELOPMENT OF ECHINODERMS.[1]

A. P. MATHEWS.

While a great deal has been done on the influence of various external factors on development, little is known concerning the influence of those various metabolic products produced by the organism itself. It is probable that such products influence both the rate and character of development and it has long seemed to me that by varying them we could most surely reach our goal of an understanding and control of the mechanism of development. Accordingly I added to sea-water containing developing eggs of the sea-urchin, *Arbacia punctulata*, various amounts of cystin, leucin and tyrosin to see what changes would be produced in development.

The most interesting result was obtained with cystin. Cystin is the sulfur-containing amino-acid of the albumin molecule and has the composition, $C_6S_2N_2H_{12}O_4$. The sample I used was obtained by Mörner's method from horn. It is almost insoluble in cold water. One hundred cubic centimeters of sea-water were shaken for a moment with about a centigram of crystalline cystin and the mixture poured into a finger bowl with the undissolved cystin. The eggs, fertilized something less than an hour before, the time varying in different experiments, were then added and the eggs lay during development among the crystals of cystin at the bottom of the dish. At the same time similar transfers from the same lot of eggs were made to sea-water shaken in the same way but without the cystin. This was to throw out the possibility that aeration or some other factor was responsible for the results obtained.

The sea-urchin eggs this summer showed again, in many instances, the remarkable peculiarity recorded by Whitcher[2] and myself, a large number of eggs while living for several days not forming plutei, or but a small per cent. of irregular plutei, and the proportion of these going abnormally was increased by the act of transfer. The effect of cystin on such eggs was indeed most striking. Whereas in the controls hardly a pluteus was to be found and these few were generally abnormal, the cystin eggs nearly all formed normal plutei. The effect, also, of the cystin on the development of the more normal eggs was perfectly invariable. The cystin eggs always showed a decided acceleration of development, so that they were plutei while the controls were still gastrulæ. The acceleration was visible from about the fourth division on. Plutei formed in the cystin solutions within 24 hours of fertilization. The cystin not only accelerates the process of pluteus formation, but a gradual acceleration of development takes place from the start.

---

[1] From the Marine Biological Laboratory, Woods Holl.

[2] Mathews and Whitcher, *Amer. Journal of Physiol.*, VIII., 1903, p. 300.

We have, therefore, in cystin a substance which acts most beneficially on *Arbacia* development, particularly in those cases where the development is for some reason abnormal. It resembles in this particular the action of alkalies as recorded by Loeb[1] and pilocarpine in the case of star-fish eggs.[2] The conclusion is not of course justified that the abnormality is due to a deficiency of cystin possibly produced normally by intracellular digestion, although such a possibility is not in itself improbable. If the eggs are left in contact with cystin their death takes place after 48 hours, but if they are transferred to fresh sea-water after 24 hours in the cystin water, their subsequent development does not appear to be interfered with. They develop normally thereafter.

Toward the star-fish eggs cystin proved to be toxic and the only effect noticed was the early death of the egg. No acceleration was produced in the development of the mollusc, *Cumingia*, by the addition of cystin. The action appears, therefore, to be rather specific for *Arbacia*.

I also tried the action of tyrosin and an impure leucin from horn upon *Arbacia* eggs. The tyrosin, while not very soluble, is more soluble than cystin and accordingly more of it is in solution to act on the eggs. A saturated solution was at first used, the sea-water always containing undissolved tyrosin crystals. The effect of such a saturated solution on the development of *Arbacia*, when the experiments were tried in the same way as those with cystin, was invariably harmful; development was retarded and the eggs ultimately killed. With weaker solutions the results were uncertain. In some cases the development was slightly accelerated, but more often it was either not affected or else retarded.

---

[1] Loeb, *Archiv für Entwickelungsmechanik*, 1898, VII., p. 631.

[2] Mathews, *Amer. Journal of Physiol.*, VI., 1901, p. 207.

Impure leucin prepared from horn proved very toxic, possibly owing to some impurity, but possibly to the leucin itself. The effect of a weak solution is extremely interesting in that it arrested development without killing the eggs. Particularly it prevented development beyond the blastula stage. The embryos were unable to escape from the membranes; they remained without farther development in these membranes but still alive, for 24 to 72 hours. The color gradually disappeared until they were as colorless as star-fish larvæ. I took some of them out to fresh sea-water after being thus blastulæ for 30 to 40 hours, to see whether their subsequent development would be interfered with. After a time the embryos emerged from the membranes and swam about; they lived for days and developed into all sorts of fantastic embryos. Some of them were totally unlike *Arbacia* larvæ. In many, evagination of the entoderm instead of invagination, took place. A few developed a ciliated band in the shape of the star-fish *bipinnaria* larvæ which they resembled far more nearly than they did *Arbacia*. These forms, however, never developed a well marked entoderm like that of the star-fish, nor did the mouth and anus invaginate. Another highly interesting form was perfectly spherical with a single ciliated band about the middle and swimming rapidly. It looked in its external form like a very small trochophore. Obviously the sample of leucin which was used checked some particular development but allowed other processes to go on so that the embryos lived but developed in a widely different direction from normal. I know of no means of proving so clearly the infinite number of different embryonic forms resident in each egg. Possibly an examination of these different forms might be of decided interest, and I hope that some embryologist may think the subject worth investigating since it lies apart from my own field. I was unable to make all the embryos abnormal in one direction by leucin, but I did not particularly investigate this possibility.

These experiments show that the products of intracellular protein digestion may be very important in determining development.

EXPERIMENTAL CONTROL OF CERTAIN REGULATORY PROCESSES IN HARENACTIS ATTENUATA.

C. M. CHILD.

In my study of form regulation in *Cerianthus*[1] it was shown that distension of the enteron with fluid was an important factor in regulation in this form. More recent work on *Harenactis attenuata* an actinian inhabiting the tide flats near San Diego, Cal.,[2] has confirmed my earlier conclusions and has afforded certain striking examples of the importance of distension for the persistence of parts. The results of my work on *Harenactis* will be presented elsewhere, but it seems desirable to give a brief account of certain experiments at this time since they illustrate so clearly not only the importance of distension but the possibility of experimental control of the course of regulation.

These experiments are concerned with the production of partial discs proximal to the original disc in consequence of cuts part way through the body. In my earlier experiments of this kind on *Cerianthus*[3] it was found that in all cases the region directly distal to the partial disc underwent atrophy and disappeared and the partial disc gradually migrated toward the distal end of the body and finally took the place of the atrophied portion. I pointed out that the atrophy in these cases was very evidently due to insufficient distension or absence of distension in the part concerned and showed that the method of closure of the wound in such cases resulted in isolation of the enteric cavity of the atrophying region both from the exterior and from other portions of the enteron. Although the entrance of water through the wall of the body is apparently possible to a greater or less extent, the usual degree of distension cannot be maintained in this way and in the absence of the functional stimulus produced by distension atrophy occurs.

---

[1] Child, "Form Regulation in *Cerianthus*," I.–IX., BIOL. BULL., Vols. V.–VIII., 1903–5.

[2] Child, "Form Regulation of *Harenactis attenuata* in Altered Environment," BIOL. BULL., Vol. XVI., No. 1, 1908.

[3] Child, "Form Regulation in *Cerianthus*, VIII., Supplementary Partial Discs and Heteromorphic Tentacles," BIOL. BULL., Vol. VIII., No. 2, 1905.

In the case of *Harenactis* the occurrence or non-occurrence of atrophy depends upon the conditions of the experiment and the result can be controlled.

The possibility of experimental control in *Harenactis* depends on the position of the mesenterial ostia in the œsophageal region. The ostia are readily seen in anæsthetized specimens opened under water. Their approximate position is indicated by the small dotted circles on each side of the œsophagus in Fig. 1. This figure represents an individual in the extended condition characteristic of the normal habitat, a vertical burrow in the sand. When the animals are kept without sand extension is never as great as in the burrow and the animal gradually becomes incapable of any considerable extension.[1] Under these conditions the ostium appears to lie much nearer the distal end than in Fig. 1. Each mesentery possesses an ostium, consequently the inter-mesenterial chambers are in direct communication with each other about the whole circumference of the œsophageal region.

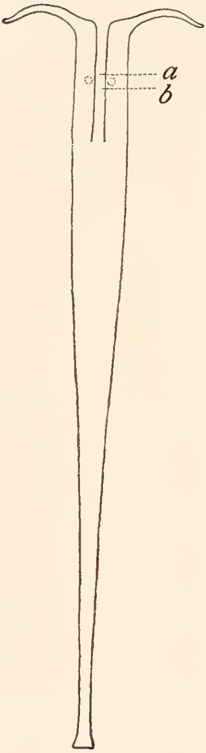


Fig. 1.

I have been unable thus far to discover the ostia in *Cerianthus* or to find references to them in the literature, so far as it is accessible to me. But whether they are present or not my experiments show very clearly that distension of the region directly distal to a lateral incision in the œsophageal region does not occur. The most casual examination of specimens after healing of the wound produced by such an operation is sufficient to show that this region is either completely collapsed or only very slightly distended.

---

[1] Child, "Form Regulation of *Harenactis attenuata* in Altered Environment," Biol. Bull., Vol. XVI., No. 1, 1908.

In *Harenactis*, as in *Cerianthus*, the healing of the wound made by a lateral incision in the œsophageal region and deep enough to involve the œsophagus occurs in the manner indicated in Fig. 2. The cut surfaces of the body-wall and the œsophagus unite with each other both distal and proximal to the cut so that a new lateral opening into the œsophagus is formed, its extent depending on the extent of the incision. The factors concerned in this method of closure will be discussed elsewhere: at present we are concerned merely with the fact of closure in this manner. After healing of the wound tentacles appear proximal to the opening, *i. e.*, on the distal end of the region below the opening, their number depending on the number of inter-mesenterial chambers which were cut across by the incision. So far *Harenactis* and *Cerianthus* are alike. In *Harenactis*, however, but not in *Cerianthus*, heteromorphic tentacles appear somewhat later at the proximal end of the region distal to the cut (Figs. 3 and 5). As will be shown elsewhere heteromorphic tentacles appear much more readily in *Harenactis* than in *Cerianthus* under various conditions. After these lateral incisions in the œsophageal region they always appear. If the old disc is removed at the time the lateral incision is made tentacles develop about the whole circumference of the distal end of the body. My figures represent cases in which the original disc was left intact: the final result is the same whether the old disc is removed or not.

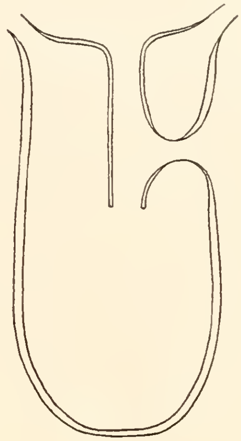


Fig. 2.

After an operation of this kind in *Harenactis* there exists then a region distal to the lateral opening in which the inter-mesenterial chambers are completely shut off from other portions of the enteric cavity in the longitudinal direction. The presence or absence of communication in the transverse direction, however, depends on the position of the incision: if this was made distal to the ostia (*a*, Fig. 1) each chamber is completely isolated from the others and from other portions of the enteron; if, on the other hand, the incision was proximal to the level of the ostia then all the chambers distal to the incision are in communication with each other and with the enteric cavity in general. In the first case distension of other portions of the body with water has no effect upon this region, but in the second, distension of other parts is accompanied by distension of this region.

In *Harenactis*, as in *Cerianthus*, a considerable degree of distension exists when the animals are undisturbed, consequently body-wall, tentacles and mesenteries are subjected to a certain degree of mechanical tension as a characteristic feature of normal life. The following experiments show very clearly the importance of this distension as a factor in determining the persistence or atrophy of parts.

Fig. 3 is a diagrammatic longitudinal section of an animal ten days after a lateral incision just distal to the level of the ostia (*a*, Fig. 1). Tentacles have developed proximal and distal to the opening as usual, but the whole region distal to the opening is much less distended than other parts of the body. The old tentacles at the distal end of this region are undergoing atrophy, as is indicated by their length as compared with the tentacles of the other side of the body and by the degenerating region at the tip: the heteromorphic tentacles at the proximal end of this region are small and short; on the other hand, the tentacles proximal to the incision are nearly as long as the old tentacles on the other side of the body.

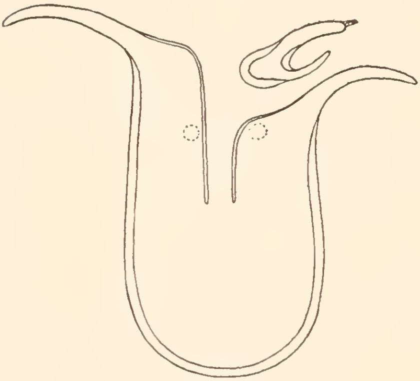


Fig. 3.

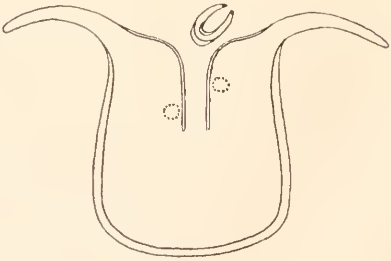


Fig. 4.

Observation of such cases in distended and collapsed condition establishes the fact that changes in the degree of distension of other regions do not alter the distension in the region directly distal to the incision. In all such cases this region acquires a certain degree of distension after healing of the wound, undoubtedly in consequence of the passage of water through the walls, but this distension gradually decreases as time goes on until finally collapse is complete.

The condition of such animals three to four weeks after the operation is indicated in Fig. 4. The region distal to the cut has undergone rapid atrophy and the partial disc below it has attained the same level as other parts of the old disc. The atrophied region is now completely collapsed and in an oral view of the animal forms merely a narrow strip across the disc with a mouth opening on each side of it.

A little later this strip breaks and its two ends become still further reduced and soon undergo complete resorption or in some cases drop off, leaving a normal disc.

The result is very different when the incision is made proximal to the level of the ostia, *e.g.*, at *b*, Fig. 1, or any level proximal to this within the œsophageal region. In such cases (Fig. 5) the old tentacles distal to the incision do not undergo atrophy and the heteromorphic tentacles become almost or quite as long as the others. Moreover, examination of the specimen shows at once that the whole region distal to the incision is as fully distended as other parts of the body when the animal is undisturbed, and when contraction and expulsion of water occurs this region takes part in the change, becoming distended again when other parts of the body distend. Fig. 5 shows an individual of this kind two weeks after the operation, a time when atrophy has already begun in cases of operation distal to the ostia. In my experiments a considerable number of these cases were kept for four and a half months. During this time decrease in size of the whole body occurred since the animals were not fed and in most cases both the oral and aboral tentacles of the region distal to the incision became somewhat shorter than the others, though there was no visible atrophy such as occurs in the other cases (Fig. 3). Fig. 6 shows an individual of this kind one hundred and thirty-six days after operation. The region distal to the incision is somewhat shorter than in the early stages but it still shares in the distension of the body, the tentacles are fully functional and the tissues appear to be in perfectly healthy condition. The termination of my stay at La Jolla made further observations impossible, but there can be little doubt that this region would have remained intact and normal almost or quite as long as other portions of the body. The fact that the tentacles of the region in question are often somewhat shorter than others is probably to be accounted for by nutritive conditions. The animals are undergoing slow starvation and apparently the material of the more proximal regions is used in greater or less degree in maintaining the more distal regions. But the region distal to the incision is only indirectly in organic connection with the proximal regions and therefore, in all probability, uses up its own material somewhat more rapidly than other portions of the body.

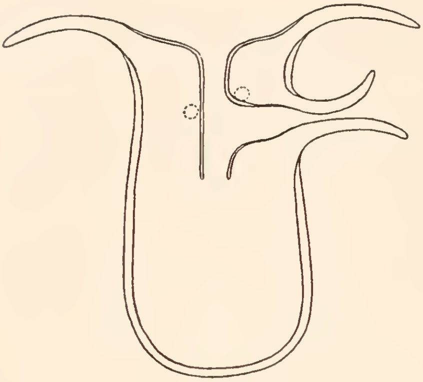


Fig. 5.

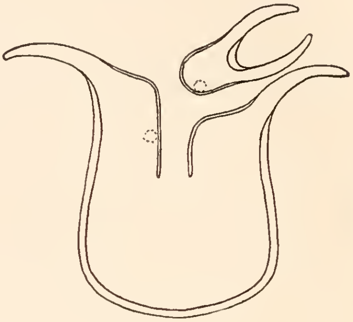


Fig. 6.

These two series of experiments seem to me to demonstrate that the conditions resulting from distension of the enteric cavity with fluid are necessary for the persistence of the body-wall and tentacles. When the incision is made distal to the level of the ostia the region distal to it cannot share in the distension of other parts and consequently undergoes complete atrophy in about a month. When the incision is made proximal to the level of the ostia this region shares in the distension of other parts and shows no appreciable degree ot atrophy within four and one half months. Discussion is unnecessary.

In a number of cases the lateral incision was made somewhat obliquely so that one end of it was distal to the level of the ostia, the other proximal. In these cases the part in which ostia were present became distended after healing, produced tentacles almost or quite as long as the others and persisted during four and one half months : the part from which ostia were absent became slightly distended after healing, produced short tentacles, then underwent gradual collapse and complete atrophy within a few weeks. Consequently these animals in their final condition possessed a structure arising from one end of the lateral incision and ending free, since the part which originally connected it with the other end of the incision had atrophied, and bearing tentacles on its oral and aboral surfaces. In certain respects these cases are the most interesting of all, since the two different results can be observed in a single animal.

To sum up : it is possible to determine experimentally whether persistence or complete atrophy of certain parts of the body of *Harenactis* shall occur : the factor determining the result is the distension of the enteric cavity with fluid or the absence of such distension.

Hull Zoological Laboratory,
University of Chicago,
December, 1908.

POLARITY AND BILATERALITY OF THE ANNELID EGG. EXPERIMENTS WITH CENTRIFUGAL FORCE.[1]

FRANK R. LILLIE.

In a previous paper (Lillie, '06) I attempted to show that the direction of polarity of the egg of *Chætopterus* is not modified by any experimental redistribution of the visible elements, nucleus and granules, and I therefore concluded that polarity is a property of the ground substance of the protoplasm. In the present paper I propose to examine the grounds for this statement, to attempt to show that bilaterality comes in the same category, and to examine the conceptions of polarity and organization that naturally result.

I. POLARITY.

Polarity manifests itself first in the egg of *Chætopterus* when the ovogonium becomes an ovocyte and takes its place in the ovarian epithelium. It has then a free pole and an attached pole, which have different physiological relations and exhibit different morphogenic activities. When the egg becomes free from the epithelium it is found that the original free pole becomes the animal pole in development, and the attached pole becomes the vegetative pole. The developmental processes take place with reference to these poles; thus the various granules of the egg rearrange themselves in a definite polarized fashion, the polar bodies have an absolutely determined relation to polarity, cleavage takes place and the various organs arise in definite topographical relations to the polarity.

In the paper already referred to I showed that if the egg is centrifuged with sufficient force the result is to separate the protoplasm into three strata, viz.: a central grayish cap, an intermediate hyaline or clear band which contains the nucleus or spindle, and a distal yellow hemisphere which contains the larger granules known as yolk (Fig. 1). The results prove that the eggs are not oriented in the centrifuge with reference to the original polarity, and therefore the axis of stratification bears all

---

[1] The substance of a part of this paper was presented before the joint session of the American Society of Zoölogists, and Section F — Zoölogy — of the American Association for the Advancement of Science, held in Chicago, December, 1907. (An abstract was printed in *Science*, Vol. XXVIII., No. 702, pp. 905–907, June 12, 1908.)

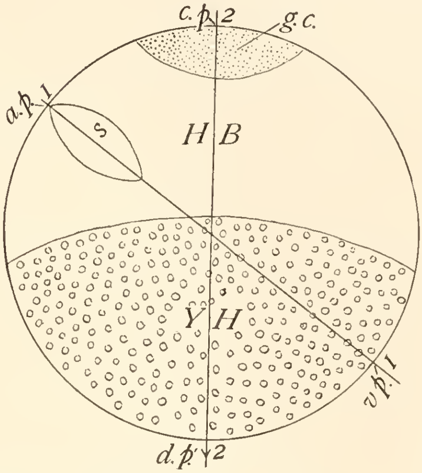


FIG. 1. Diagram of a centrifuged egg of *Chætopterus*. *a.p.*, animal pole; *c.p.*, central pole, or pole turned towards the axis of the centrifuge; *d.p.*, distal pole, or pole turned away from the axis of the centrifuge; *g.c.*, gray cap; *H.B.*, hyaline or clear band; *s.*, maturation spindle; *v.p.*, vegetative pole; *Y.H.*, yellow, yolk-bearing, or distal hemisphere; *1–1*, primary axis; *2–2*, secondary axis.

possible relations to the polar axis of the egg. We may call the original polar axis the primary axis, and the line through the center of the egg in the direction of the centrifugal force the secondary axis (Fig. 1).

If such eggs are fertilized the polar globules of different eggs form at any point of the surface with reference to the secondary axis. Cleavage takes place with reference to the axis defined by the polar globules, as in the normal egg, and is approximately normal in form. It therefore follows that the distribution of yolk and other substances induced by the centrifuge is substantially immaterial so far as the form of cleavage is concerned. The effect of abnormal distribution of yolk and other substances on the differentiation of organs is not considered here.

On the basis of these results I concluded that the polarity of the ovum had not been affected by centrifuging, and that the polar bodies appear in the same position that they would have normally, that is, in the primary axis; in other words, that no redistribution of the granules or nucleus affects the direction of polarity of the egg. If this be true, then the final conclusion stated in that paper, that polarity is a function or property of the ground substance of the protoplasm, certainly follows.

This conclusion is of fundamental importance for the entire theory of development, and its basis therefore requires the most careful examination. One thing is clear at the start, viz.: that this point of view affords a complete explanation of the phenomena, because the egg is polarized prior to centrifuging, and the centrifugal force acts in all possible directions with reference to the polar axis;[1] and this agrees with the fact that the polarity that appears after centrifuging also lies in all possible directions with reference to the axis of stratification. The question is simply this: Is the polarity as marked by the polar bodies and cleavage planes the same polarity in the normal egg and after centrifuging? Does any other hypothesis agree so well with the observed facts, and if so, is it for any sufficient reason to be preferred to this hypothesis?

An alternative hypothesis can lie only in one direction, viz.: that polarity is determined by any point on the surface of the egg to which the maturation spindle happens to go after centrifuging. Now, assuming that there is no polarity of the ground substance, as this hypothesis requires, the spindle would naturally follow the shortest path to the surface, for this would usually be the path of least resistance. In other words, the position given the nucleus or the spindle by the centrifugal force would determine the polarity in all cases, because its position is always excentric. However, this is not the case, as the following facts demonstrate :

---

[1] The proof of this statement is as follows: In the stage used for the experiments (except where otherwise stated) the first maturation spindle is fixed at the periphery at the stage of the mesophase. Its outer pole marks the center of the animal pole; this point is also characterized by an entire absence of the ectoplasm, which covers the remainder of the surface of the egg as described in a previous paper ('06). If sections of centrifuged eggs are examined, the maturation spindle is found in about half of the eggs still attached to the surface, at any point in the hemisphere containing the hyaline band. At first sight this would indicate that the eggs oriented themselves in the centrifuge to a slight extent, though it is clear that the animal pole is in some cases 90° removed from the ends of the secondary axis. But the fact that fixed spindles are not found in the distal (yolk-laden) hemisphere after centrifuging has another explanation: about half of the eggs after centrifuging have free spindles (*i. e.*, not attached to the surface), which always lie in the hyaline band, and these represent those eggs whose original animal pole lay in the distal hemisphere in the centrifuge. (Cf. Fig. 8, *C* and *D*.) The inrush of yolk into the distal hemisphere has torn the spindles loose from the periphery, and their specific gravity has carried them into the hyaline band; this interpretation of the free spindles is proved by the fact that many of them migrate into the distal hemisphere later in the process of forming the polar bodies (Fig. 2).

1. Most of the experiments on the egg of *Chætopterus* were made before fertilization at the stage of the mesophase of the first maturation spindle. All the ripe eggs of this animal attain this stage without fertilization, and the spindle comes to rest and remains at the mesophase unless the egg be fertilized or otherwise effectively stimulated (Fig. 4). The spindle is attached to the surface and its axis is in line with the axis of the egg. If the direction of the centrifugal force is such that the spindle lies in the central hemisphere, *i. e.*, in the half of the egg towards the post, it may remain attached, but is often detached from the surface, and in such cases it always lies in the clear band. If the spindle pole of the egg lies in the distal hemisphere during centrifuging, it is always detached from the surface and comes to rest in the clear band. The result therefore is always the same so far as the position of the spindle is concerned : the spindle lies invariably in the clear band after centrifuging, either attached to the surface, or free.

Nevertheless, the polar bodies may form at any point in the yolk hemisphere, as is demonstrated by study of the living eggs, and also by sections of series of eggs preserved during the maturation period after centrifuging. Figures of such living eggs are given in the paper already referred to, and a section of such an egg is reproduced here (Fig. 2). When one considers the resistance that has to be encountered by the spindle in making its way through the densely packed yolk-granules of the distal hemisphere, and remembers that a shorter path to the surface through hyaline protoplasm was open, it is obvious not only that the spindle does not follow the shortest path to the surface, but also that a factor of great intensity has been in operation.

I stated in my previous paper ('06) that the formation of polar globules in the yolk hemisphere is rarer than their formation in the hyaline hemisphere, and was inclined to regard this as evidence that there was a tendency for the eggs to orient themselves in the centrifuge with the vegetative pole away from the center. But I no longer believe that this is the case. The relative rarity of maturation at the distal pole of centrifuged eggs must be due to a different cause, and this is the great resistance of the yolk mass to penetration by the spindle. This conclusion is clearly demonstrated by study of series of sections of eggs preserved at regular intervals after centrifuging and fertilizing, which show that many such eggs fail to form the polar bodies at all, or are much delayed, or undergo their maturation divisions within the egg.

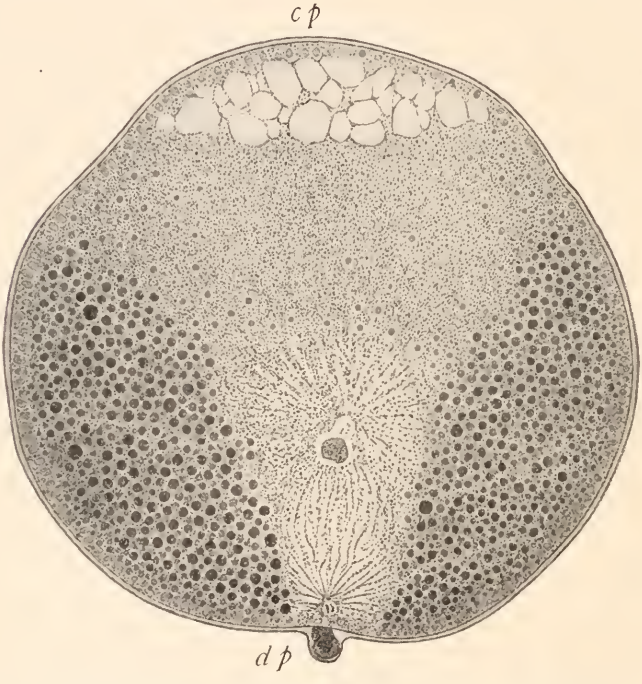


FIG. 2. Egg of *Chætopterus* centrifuged 2,300 revolutions in one minute at a radius of 13 cm., 3 : 57 P. M., June 27, 1906; fertilized 3 : 58 P. M., killed 4 : 27 P. M. The formation of the polar globules has taken place at the distal pole. The maturation spindle migrated through the entire yolk-mass in the distal hemisphere to reach this position. The sperm-nucleus surrounded by radiations is seen a short distance above the egg-nucleus. The vacuolated area above is the gray cap. *c.p.*, central pole (secondary axis); *d.p.*, distal pole (secondary axis).

2. An even more conclusive argument is afforded by centrifuging ovocytes with intact germinal vesicle. As shown in the paper already referred to, the large germinal vesicle passes to the central pole; it is the lightest constituent of the egg. If, therefore, the position of the nucleus determines polarity, the central pole should become the animal pole in such cases; the polar bodies should form here. I made a number of experiments on this subject in 1906 and 1908. The difficulties found in interpreting these experiments are: (*a*) that the stratification induced by low centrifugal powers is relatively slight in the stage with intact germinal vesicle, apparently owing to greater vicosity of the ground substance at this stage, and (*b*) that it does not persist to the time of formation of the polar bodies, owing to the process of polarization of granules that follows the rupture of the germinal vesicle, which I described in a previous paper. However, by following individual eggs in which the position of the centrifuged germinal vesicle is known, the relation of the polar bodies to the position of the germinal vesicle can be readily ascertained. It is then found that there is no fixity of relation whatever; in other words, the maturation spindle, which forms at the site of the germinal vesicle, may migrate through the entire diameter of the egg to become fixed at the opposite pole, or perform any lesser migration. The same thing is demonstrated if the eggs are submitted to very high centrifugal powers; under these circumstances the stratification persists up to maturation in some eggs to a sufficient degree to demonstrate positively that the polar bodies form in all positions with reference to the centrifuged position of the germinal vesicle.

Thus one cannot determine the place of formation of the polar bodies by simply throwing the nucleus to the surface of the egg.

A third argument may be based on comparative grounds. Very many cases are known in which polarity determines the position of the nucleus; the literature on fertilization is full of such cases. I know of no observations that tend to show determination of polarity by position of the nucleus.

The results, then, certainly demonstrate that polarity is not a result of the position of the nucleus or of any configuration of granules. It follows that it is a property of the ground substance; and when one considers carefully the data presented, one cannot avoid the conclusion that the direction of polarity is unmodified by the centrifugal powers employed. It must therefore depend on some configuration or heterogeneous physical or chemical properties of the ground substance established early in the history of the egg, and which is not essentially disturbed by centrifuging. This subject will be examined in the third part of the present paper.

The conclusion stated above was clearly presented in my paper of 1906. Since then Morgan (1908) has come to the same conclusion for the egg of *Arbacia.* In his last paper Morgan furnishes a satisfactory demonstration that polarity is unmodified in the egg of *Arbacia* by centrifuging, by showing that the micropyle which bears a constant relation to polarity in the normal egg has the same constant relations to the polarity of centrifuged eggs, as proved by formation of the micromeres and archenteron at the pole opposite the micropyle whatever the direction of the centrifugal force, and therefore whatever the position of the centrifugal zones and the form of the cleavage modified (in *Arbacia*) by these zones. I therefore take Morgan's result to be a complete confirmation of my original and present position.

The demonstration that the direction of polarity is unaltered by centrifuging involves the assumption that the configuration of the ground substance remains the same. In other words, that there is a definite architecture in the ground substance, which is the basis of the localization pattern in normal development (see *Science*, N. S., XXVII., June 12, 1908). This also involves a new conception of protoplasmic streaming as seen in ova, for this must be interpreted as granule movement exclusively, and not actual flowing. Similarly, the movements produced by the centrifuge cannot be mass movements of entire protoplasmic areas, but only granule movements through the ground substance. In the third part of the present paper, I shall consider these conclusions with some care.

Observations on Nereis.

In the summer of 1908, I had the opportunity of comparing the eggs of *Nereis limbata* with reference to these points, and I found that, just as in *Chætopterus*, the place of formation of the polar globules is independent of centrifugal stratification, and that the cleavage follows the polarity marked by the polar globules and is not modified in its direction by the position of the yolk arbitrarily imposed by the centrifuge. Morgan has shown the same thing for the lamellibranch, *Cumingia* (*Science*, Vol. XXVII., p. 496, 1908), so that now we have at least four forms in which this principle is firmly established.

II. Bilaterality.

The second main question that I propose is whether or not bilaterality comes under the same category as polarity. And this resolves itself into two questions: (1) Is bilaterality, like polarity, a function of the ground substance, and (2) When does it arise?

1. The first morphological evidence of bilaterality in the eggs of *Chætopterus* and *Nereis* is found in the first cleavage. The spindle takes an excentric position, and the cleavage is therefore unequal; the smaller cell is anterior and the larger cell posterior in position. The course of the entire development is thus determined not only with reference to the bilateral axis, but also with reference to the proportions of embryonic organs.

If the bilaterality were a result of a kind of equilibrium of the protoplasmic inclusions (nucleus and granules), it is clear that the variations in position of the nucleus and granules produced by centrifugal force should involve change of direction of bilaterality. If, on the other hand, bilaterality is a function of the ground substance, changes in the position of the nucleus and granules should not materially affect the direction of bilaterality, though it might conceivably modify the inequality of the cleavage which is our index of bilateral organization. However, if the normal proportions of the first two cells and the meridional direction of the cleavage plane are maintained in centrifuged eggs in spite of the abnormal distribution of granules between cells that would be thus induced, no other evidence would be necessary to show that the seat of bilateral organization is in the ground substance.

This is in fact the case, both in *Chætopterus* and *Nereis*: Whatever the distribution of yolk and granules induced by centrifuging, with reference to the primary axis, the first cleavage is invariably truly meridional, and the proportions of the cells are usually normal or approximately so. In some cases the small cell is packed full of yolk granules, in other cases practically devoid of them ; nevertheless, the first cleavage is substantially unaffected as to direction or proportions by the direction of stratification (see Lillie, 1906, p. 199).

2. Is the bilateral organization of the egg a property of the primary ovocyte like polarity, or does it arise in the course of development after rupture of the germinal vesicle? The evidence seems to me in favor of the latter proposition for the following reasons :

(*a*) If the ground substance is bilaterally organized before maturation there should be morphological evidence of it, as there is of the existing polarity. But there is none whatever ; the structure of the egg is radially symmetrical in the plane at right angles to the polar axis until the close of maturation. After the union of the germ nuclei, the bilateral symmetry comes to expression slowly by granule movements that cause a preponderant distribution of the yolk granules on one side of the egg, which becomes the posterior side, and by corresponding displacement of the yolk-free protoplasm and spindle towards the opposite side of the egg, which becomes the anterior side. This is the case in both *Chætopterus* and *Nereis*, as well as in *Unio*. If the conditions causing such displacement existed prior to maturation, one would expect that they would become effective at an earlier period.

(*b*) In the summer of 1908 I found that it was possible to break up the eggs of *Chætopterus* into fragments of varying sizes by high centrifugal force. The protoplasm of this egg is much more fluid than that of any other egg I have tested ; in consequence, the egg elongates even with relatively low centrifugal speed ; and with very high speed the eggs break apart along the lines of stratification. Thus one obtains numerous perfectly hyaline pieces composed of the substance of the clear band, and others densely packed with yolk granules. The nucleus is almost invariably contained in the former kind, but it may occur in the latter, owing to the fact that at the time of centrifuging the matu-

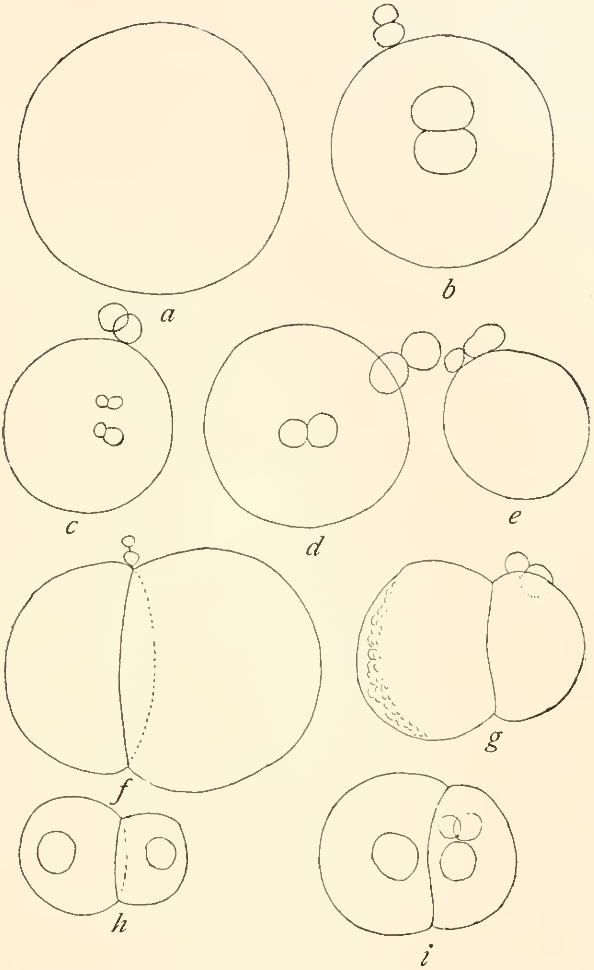


FIG. 3. Maturation and first division of hyaline fragments of the eggs of *Chætopterus*, obtained by centrifuging before fertilization. *a*, outline of normal egg for size-control ; *f*, outline of normal two-celled stage for size-control ; *b*, *c*, *d*, *e*, *g*, and *h*, hyaline fragments of various sizes from a single experiment ; *i*, a similar fragment from another experiment. Fragment *g* contains a small quantity of yolk.

The eggs break apart along the planes of stratification with a high centrifugal force, and further subdivision may occur so that the fragments are variable in size.

It will be noted that the polar globules are abnormally large in the fragments (cf. Fig. *f*, control). The proportions of the first two cells are, however, approximately normal, Figs. *g*, *h*, *i* (cf. *f*, control).

All figures drawn with the camera at a magnification of 780 diameters, and reduced one half.

ration spindle is attached to the surface, and may thus be separated off in the yolk-bearing end of the centrifuged egg, if it lies in a distal direction in the centrifuge and is not torn loose from its attachment before rupture of the egg takes place. But while the majority of large hyaline pieces contain the maturation spindle and form polar bodies after fertilization, this happens very rarely indeed in yolk-bearing pieces.

I was interested to determine if the cleavage of the hyaline pieces was unequal, and, if so, whether or not the proportions of the cells approximated the normal. This is indeed the case ; in most pieces taken before fertilization, that segment, the segmentation is unequal and in substantially the normal proportions. Seeing that the inequality of cleavage of the normal egg is an unfailing index of bilaterality, the same phenomenon in the parts must be interpreted in the same way.

The unequal cleavage of such hyaline parts absolutely devoid of yolk is a most striking phenomenon. There is great variation in the absolute size of such parts, but the cleavage remains proportional.

The parts vary not only in size, but also in the regions of the egg that they represent. The latter conclusion obviously follows from the fact that the direction of the centrifugal force is a matter of chance.

To show the strong tendency of pieces of the hyaline band to segment in normal proportions the following result may be cited. In experiment 10 *F*, August 18, 1908, a quantity of eggs taken from the female at 10 A. M. was allowed to stand in sea water without fertilization until 10 : 40 A. M. They were then centrifuged about 7,500 revolutions in one minute with a radius of 6 cm. and were thus broken up. The material was fertilized at 10 : 43 and allowed to develop to the two-celled stage, then fixed, stained and mounted entire in balsam. The impression of the preparation is that in practically every segmented hyaline piece the proportions of the cells are approximately normal. To remove the personal equation as far as possible, I marked a ring on the cover slip, and drew every segmented hyaline piece in it. Of 26 pieces so drawn, none had divided equally ; in four pieces of the 26 the small cell was proportionally larger than normal, and the remainder were substantially normal in the relative sizes of the two cells, although the smallest piece was about one eighth the volume of the entire egg, and the others ranged in size up to about one third the bulk of the normal egg. The pieces in which the small cell was too large relatively were the largest pieces and contained some yolk.

Now if bilateral symmetry existed prior to fertilization, one would expect parts of the bilateral egg to vary greatly in the proportions of the first two cells, because a fractional bilaterality and not a whole bilaterality would exist in each part. I therefore conclude that bilaterality has developed subsequent to fertilization. And this conclusion must be true *for the parts* even if it were only a question of regulation of a preëxisting bilaterality (of which there is no evidence). But if bilaterality can develop in the parts there is no reason for assuming its prior existence in the whole ; and its origin must be regarded as a truly epigenetic process. Brachet makes a similar argument for the egg of the frog (*Roux's Archiv*, XXII., 3, 1906).

III. THE ORGANIZATION IN THE GROUND SUBSTANCE. EXAMINATION OF THE CONCEPTIONS OF POLARITY AND BILATERALITY.

The hypothesis that the nature and direction of polarity and bilaterality are unaltered by centrifuging involves the assumption that the arrangement of the ground substance is substantially unaltered. If it could be shown, for instance, that the segregation produced by centrifuging is a mass movement of the areas and zones of the protoplasm, and not simply a movement of granules through the ground substance, the entire organization of the egg would be altered, and there would be no reason why polarity should appear in the original direction rather than in any other direction.

I propose therefore to examine the thesis that the movements produced by centrifuging are purely granule movements, with the single exception of the nucleus or spindle. To do this it is necessary to compare minutely the structure of the egg both before and after centrifuging, using a fixed and determinate stage for such examination. Fortunately, the latter condition is a relatively simple matter in the case of the egg of *Chætopterus*, because all ripe eggs reach such a stage in about twenty minutes after they are put in sea water, and undergo no further progressive development unless fertilized or otherwise suitably stimulated.

This stage may be described as follows (Fig. 4):

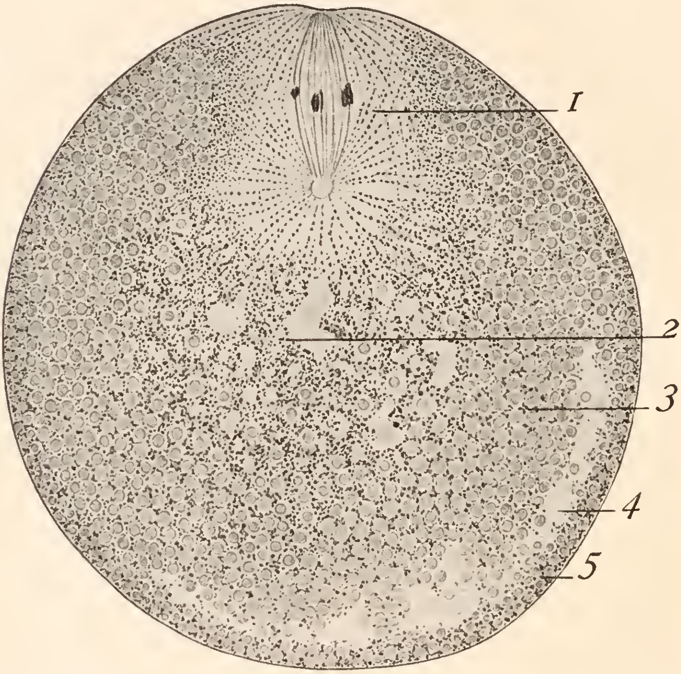


FIG. 4. Section of the egg of *Chætopterus* at the stage used for the experiments. See text for description.

The substance of the polarized egg shows the following areas and zones:

1. *The spindle area* lies at the animal pole and includes the spindle and the protoplasm in which the astral rays are developed. This area is predominantly basophile, but it may include a very few acidophile yolk granules. The astral rays may encroach slightly on (2) and (3).

2. *The central spongy area* lies near the center of the egg in the direct line of prolongation of the spindle. Its spongy appearance is due to paucity of granules and not to actual vacuoli-

zation of the ground substance. The basophile granules predominate and are arranged in a loose spongy network. This area is bounded by the spindle area on the animal pole side, and by the spherular zone (3) on the other sides. The boundary towards the spindle area is a little indefinite, fairly definite on the other sides. Its form is that of an indented sphere, the indentation being produced by the spindle area. Its position is about the same as that of the germinal vesicle before rupture.

Together, the spindle area and the central spongy area form an ovoidal mass, the small end of which reaches the surface at the animal pole, while the large end extends below the center of the egg.

The other zones of the egg are arranged concentrically to this mass, and are therefore open at the animal pole and thickest at the sides and vegetative end.

3. *The spherular zone* is so named because it contains the major part of the endoplasmic spherules or endoplasmic acidophile granules (yolk). In section, it appears as a very deep gourd-shaped crescent. The thin horns reach up above the level of the equatorial plate of the spindle.

4. *The intermediate spongy zone* lies between (4) and (5). In many eggs it is barely noticeable, in others it is very prominent. Like the central spongy zone, it is not a vacuolated portion of the ground substance, but it is characterized by paucity of granules, and so appears unstained in sections. It is a genuine structural feature as is proved by experiments, and also by the fact that it becomes more pronounced in eggs allowed to remain in the sea water (Fig. 5).

5. *The ectoplasmic zone* is the peripheral boundary of the egg, except that it is defective at the animal pole where the spindle area comes to the surface. It is characterized by the presence of spherules that differ in certain properties from those of the spherular zone (endoplasm), as described in detail in a previous paper.

In eggs allowed to stand in sea water without fertilization these areas become more strongly differentiated (Fig. 5), and the fact that the egg has a concentric as well as a polar organization stands out more strongly. The central spongy area now appears to have a thick and dense wall in which the spherules of the spherular zone are especially aggregated. The intermediate spongy zone appears wider. The striking similarity of this configuration to that produced by low centrifugal powers will be seen as the description proceeds.

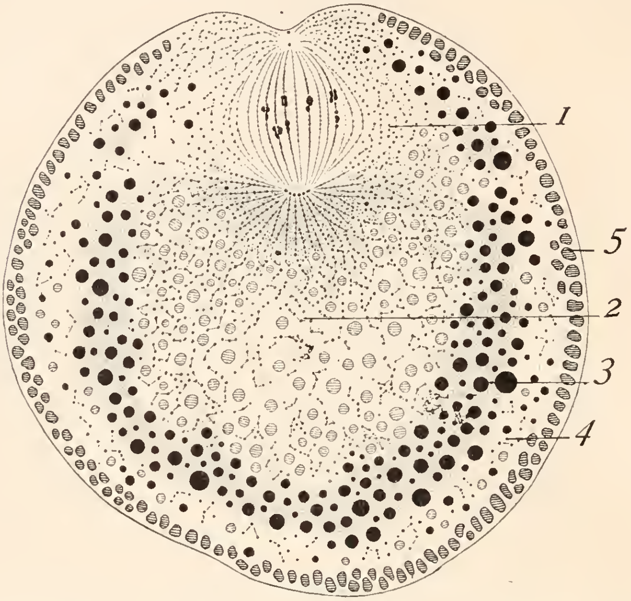


FIG. 5. Section of an unfertilized egg of *Chætopterus* that stood in sea water two hours and seven minutes before killing.

We may now compare the pictures presented by eggs of this stage that have been centrifuged at different speeds: For this purpose we shall select first an egg that was centrifuged 1,150 revolutions in 31 seconds at a radius of 13 centimeters (Fig. 6). Comparison of a large number of eggs shows first that the position of the eggs in the centrifuge is a matter of chance and second that the segregation produced is essentially the same in whatever direction the centrifugal force may act with reference to the polar axis. We may therefore leave the question of direction of centrifugal force out of account. As already noted, the effect on the living egg is very striking: it appears stratified in the direction of action of the centrifugal force; at the central pole is a small gray cap, next to it a broad, clear or hyaline band, and the distal hemisphere appears intensely yellow, and packed full of yolk-granules. The surface of apposition of the hyaline band and yellow hemisphere is not a plane surface, but decidedly convex towards the hyaline band.

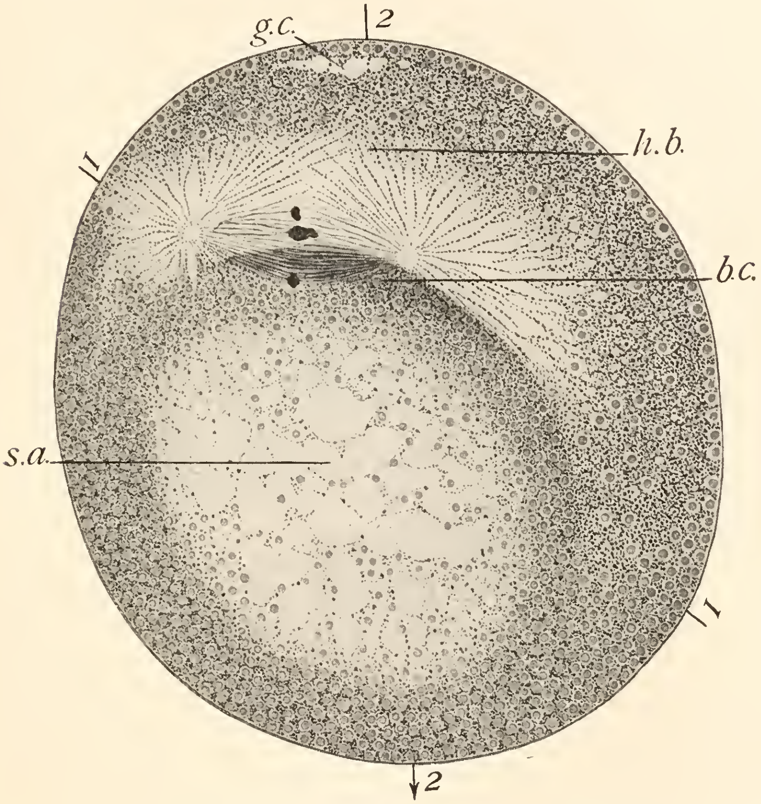


FIG. 6. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. The force was not sufficient to produce a very full segregation of the material of the gray cap. *1–1*, primary axis of the egg ; *2–2*, secondary axis. *b.c.*, basophile cap ; *g.c.*, gray cap ; *h.b.*, hyaline band ; *s.a.*, spongy area ; stained in thionin and orange *G*.

The gray cap is composed of fat globules, as is demonstrated by staining with osmic acid (Fig. 9) ; whether or not it includes the residual substance of the germinal vesicle as maintained in my former paper, I am not now prepared to say. In *Nereis*, in place of the gray cap, we have an aggregation of the large oil drops characteristic of this egg. The hyaline band contains the small basophile granules, and the distal hemisphere the large acidophile granules (yolk-spheres). In sections stained in thionin and orange *G* the hyaline band is bright blue and very finely granular; the distal hemisphere is orange, owing to the affinity of the large yolk granules for this stain. But among the orange spherules are some basophile granules, and with the centrifugal power noted above, some orange spherules may be found in the basophile band. But with a sufficiently high power a practically complete separation of basophile and acidophile granules may be brought about.

Attention must now be directed to a definite configuration in the distal or yellow hemisphere, viz., a clear, spongy area which extends into the base of the clear band, and which is surrounded distally and laterally by acidophile granules. It is similar to the central spongy area of the normal control eggs with which it must be identified. It contains relatively few granules, which are arranged in a coarse meshwork. The central end of this area is occupied by an aggregation of basophile granules which are so much more densely packed than those in the clear band, with which the special aggregation is continuous, as to contrast strikingly with it in sections; particularly with low powers of the microscope. The form of this aggregation is lunate or biconvex in section, thick in the center and diminishing towards the ends.

The large acidophile granules tend in a distal direction and the smaller basophlle granules in a central direction. If there were no differences in resistance in the ground substance they would pass in straight lines. But the fact is that they do not do so, and this is *prima facie* evidence of differences in resistance of the ground substance in different areas:

The basophile cap in the central boundary of the spongy area is a clear demonstration of this principle: In the first place it is an aggregation of basophile granules; this is proved by the staining reaction, always basic. That the stain is in the granules and not in the ground substance is readily demonstrated in thin sections, and the granules are of the same size as the basic granules of the clear band. In the second place, since such basophile granules tend to move centrally, that is, towards the hyaline band, under the influence of centrifugal force, this aggregation, which does not exist in the control eggs, must have come from the distal hemisphere. But as they lie at the central end of the spongy area, and are most numerous opposite the center of this area, and diminish towards the sides, it is obvious that they must have come from the interior of this area.

They are in fact a swarm of microsomes moving under the influence of centrifugal force, but delayed on the borderland of the hyaline band (cf. Fig. 7), where they belong by virtue of their specific gravity, by the greater density (or other mode of resistance) of the wall of the spongy area.

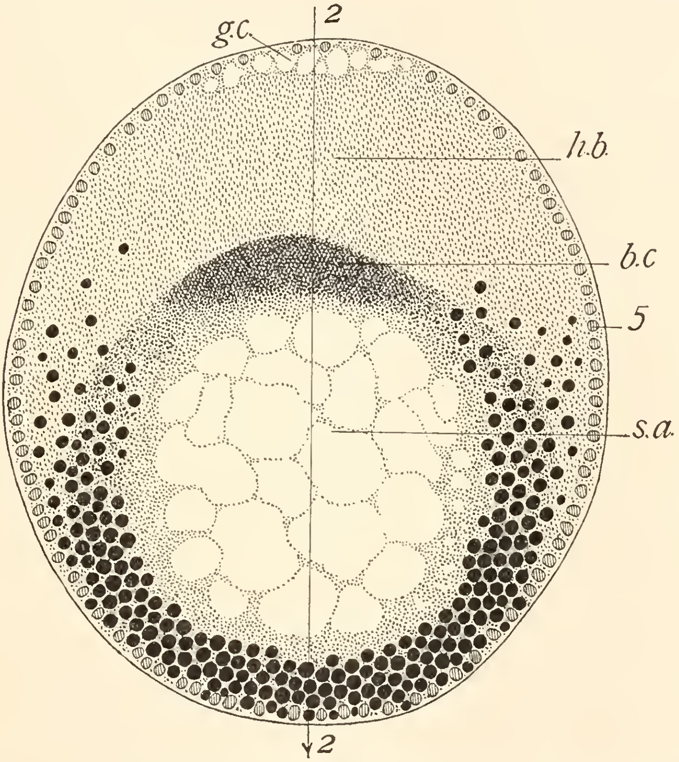


FIG. 7. Section of an egg of *Chætopterus* centrifuged 1,150 revolutions in 31 seconds at a radius of 13 cm. The primary axis of the egg is unknown in this case. *2–2*, secondary axis; *5*, ectoplasmic zone; *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area; stained in iron hæmatoxylin and orange *G*.

This property of the wall of the spongy area also explains the form of aggregation of the acidophile granules, which tend on this account to pass around and not into it. It also explains the more striking character of the spongy area in the centrifuged and the control eggs (cf. Figs. 6 and 4), because the granules within this area are free to segregate at its ends, but those without are prevented from entering.

Similarly, the persistence of the granules in the ectoplasmic zone indicates that this is another region of greater density of the ground substance. It requires a much greater centrifugal force to dislodge these granules than those lying within the egg, but when they are so dislodged they appear to be the heaviest class of granules within the egg, for they aggregrate at the distal pole (Fig. 9).

The following diagrams (Fig. 8) illustrate both the conception of the ground substance to which I have come as a result of the experiments, and the effect of the centrifugal force on the ground substance itself: The latter consists of four concentric layers, 2, 3, 4 and 5 (Figs. *A* and *C*); of which 5, the ectoplasmic stratum, is relatively dense, 4, the zone containing most of the acidophile granules of the normal egg is relatively fluid, 3, again, is a dense ring-shaped zone, surrounding 2, the central, more fluid spongy area.

The egg is an elastic sphere; it therefore undergoes elongation in the direction of the centrifugal force (Fig. 8, *B* and *D*); this is perfectly obvious immediately after centrifuging. The arrows in the figures to the left indicate the direction of the centrifugal force and the figure to the right in each case represents the result. Now observation shows that the spongy area of centrifuged eggs lies in the distal hemisphere, hence elongation of the ground substance must have taken place on the central side, owing presumably to the tension of the specifically very light substances (*e. g.*, fat globules) found here.

I have employed five or six different degrees of centrifugal force in examining this question and have made a considerable number of experiments with each. The configuration which we have just described varies somewhat with different degrees. With less force the same configuration is found down to 575 revolutions in 17 seconds with a radius of 13 cm. With higher forces it tends gradually to disappear as is to be expected, for it is obvious that with a sufficient force the granules would be driven through or out of areas of greater density very readily and would segregate solely with reference to their specific gravity. With a speed of 7,500 revolutions in one minute at a radius of about 6 centimeters, one can hardly observe any trace of the concentric arrangement (Fig. 9). Abundance of proof can be furnished for

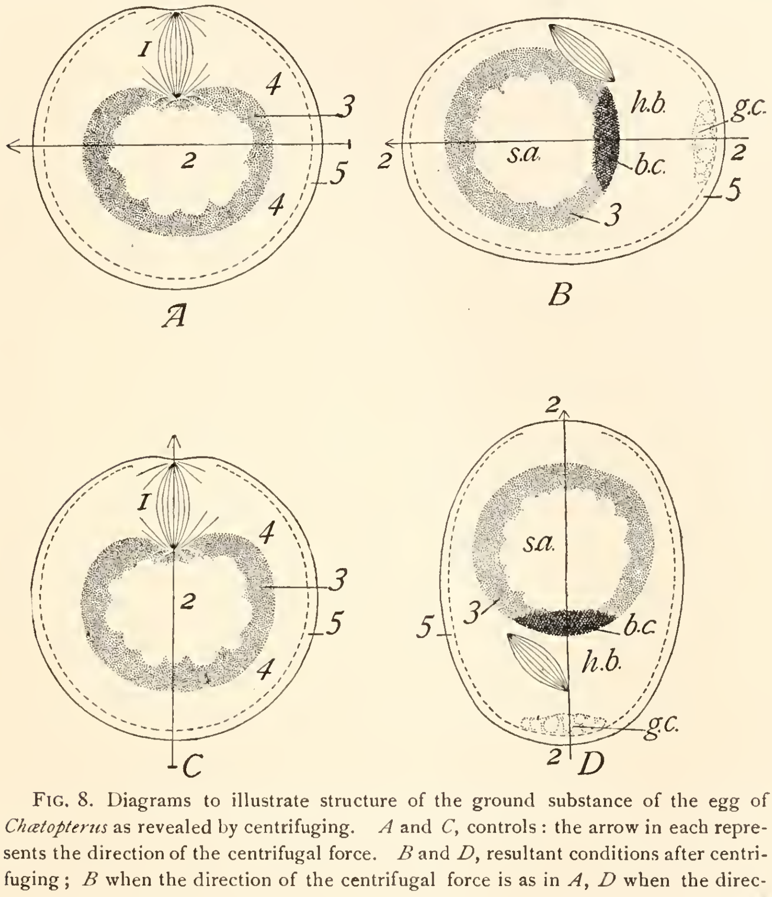


FIG. 8. Diagrams to illustrate structure of the ground substance of the egg of *Chætopterus* as revealed by centrifuging. *A* and *C*, controls: the arrow in each represents the direction of the centrifugal force. *B* and *D*, resultant conditions after centrifuging; *B* when the direction of the centrifugal force is as in *A*, *D* when the direction of the centrifugal force is as in *C*. *1*, spindle area; *2*, spongy area of control eggs; *2–2*, secondary axis of centrifuged eggs; *3*, dense ring-shaped zone in the ground substance; *4*, relatively fluid zone; *5*, ectoplasmic zone. The numbers have about the same significance as in Fig. 4. *b.c.*, basophile cap; *g.c.*, gray cap; *h.b.*, hyaline band; *s.a.*, spongy area of centrifuged eggs.

these statements, but there would not be much value in multiplying instances by describing all of the intermediate conditions.

The same configuration is found in all stages of maturation and fertilization up to the first cleavage, at which time the cleavage naturally causes modification. Whether or not it reappears in each cell is a question that I have not examined.

It may be objected that the configuration shown could be produced as readily by mass movements of the protoplasmic areas as by individual granule movements. But such a conception would meet a fatal difficulty in the case of the central spongy area ; the basophile cap in the central end (with reference to the centrifuge) of this area could not be produced in any other way than by a granule movement. Moreover, detailed examination of the movements in the spherular zone (Fig. 4, *3*) seem to me to be equally conclusive : If the movements produced by centrifugal force in this area were mass movements of the protoplasm as a whole the conformation of this zone should vary with the direction of the centrifugal force until it reached a final position of equilibrium, for the reason that it would fold in different ways around the central spongy area. The graded series of centrifugal forces employed may be regarded as showing stages in the segregation of the granules of this area. If the movements were mass movements the animal pole extensions of the spherular zone (Fig. 4) must wrap around the central spongy area where the centrifugal force acts at any appreciable angle from the egg-axis. But this is not the case ; the proximal pole of the spongy area is never covered by such a fold. With the lowest speed, in directions at any appreciable angle to the egg-axis, one finds that the hyaline zone is dotted with granules of the spherular zone, evidently caught in migration ; such granules are rarer with successively higher powers of the centrifuge, until they are practically absent. It is obvious that they pass individually across the hyaline zone.

Another conclusive proof of individual granule movements is shown in Fig. 9. The spindle is in a very high degree resistant to penetration, and it therefore often acts as a block to migrating granules, which pile up against it. The figure illustrates the blocking of fat granules which are for the most part accumulated at the central end of the egg (upper end in the figure). Innumerable cases of this kind are found in the preparations.

Again, as already noted, the ectoplasmic granules tend to keep within the ectoplasmic zone with low powers of the centrifuge.

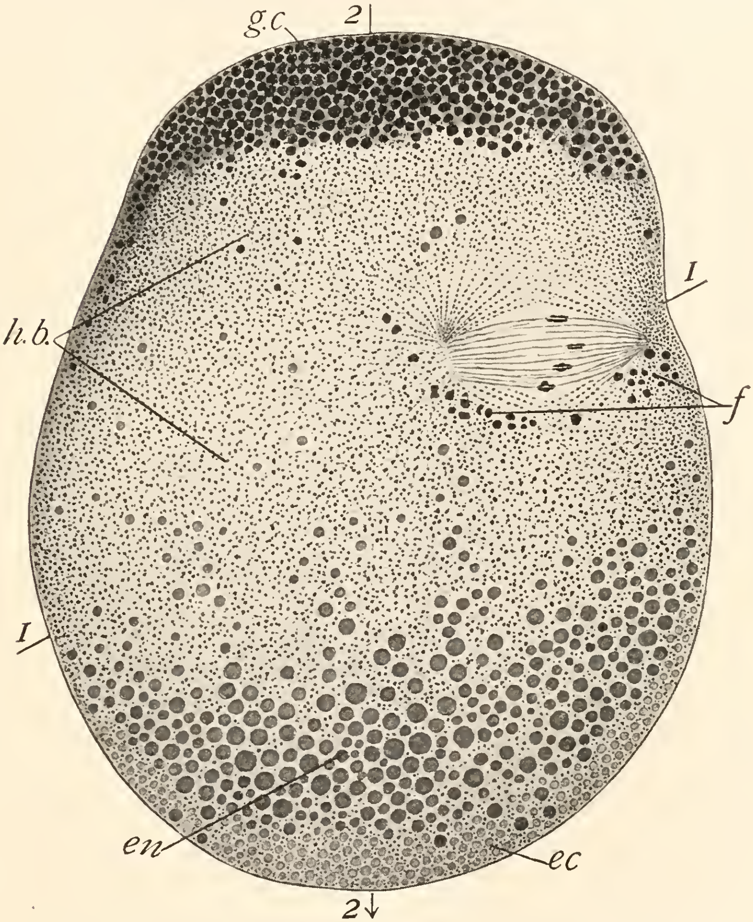


FIG. 9. Section of an egg of *Chætopterus* centrifuged 7,800 revolutions in one minute at a radius of 6 cm. The egg was killed in Flemming's fluid, and the fat granules of the gray cap are stained black. The spindle blocks fat globules passing in a central direction (*f*). *1–1*, primary axis; *2–2*, secondary axis; *ec.*, ectoplasmic spherules; *en.*, endoplasmic spherules in the distal hemisphere; *f.*, fat globules blocked by the spindle, *g.c.*, gray cap; *h.b.*, hyaline band.

But with higher powers they pass to the distal pole of the egg. Nevertheless the ectoplasmic zone may be recognized without the granules, after fixation with Flemming's fluid, as shown in Fig. 9.

The conclusion of this whole matter is that there is demonstrative evidence of individual movement of all classes of granules under centrifugal power, and no evidence of mass movements of protoplasmic areas. These movements reveal a definite form of concentric organization in the ground substance, which is only indistinctly revealed by the granule arrangement seen in the normal egg.

It does not require much argument to show that the organization of the ground substance is the primary factor that determines the concentric arrangement of the granules. The idea that the organization in the ground substance is secondarily produced by a given arrangement of granules is contrary to the entire series of results contained in this paper.

The granules reach their position and form the normal segregation pattern by "flowing" movements. Unless the entire argument of the present paper is incorrect, the flowing movements are simply granule movements within a firmly organized ground substance, determined by unknown factors involved in the relations of nucleus and granules to each other and to the ground substance.

It is incredible to me that vital organization can be bound up in a flux of all protoplasmic constituents. Vital processes must be bound up with some firm condition of aggregation in the protoplasm.

But how, it may be asked, are polarity and bilaterality connected with the concentric organization of the ground substance revealed by centrifuging? The answer to this question is not at all obvious. These properties are shown by the experiments to be properties of the ground substance; and the experiments further show, as illustrated in Fig. 8, that the ground substance is merely distorted, not essentially altered by centrifugal force, and this furnishes a physical basis for the persistence of polarity and bilaterality in the original directions. But their nature is not in the least revealed by the experiments. The results only furnish a partial answer to the old question, how life and organization can exist in a total flux of materials, by showing that the flux is only partial.

Morgan has made the suggestion (*Science*, August 28, 1908, p. 288) that formative substances other than the visible granules here referred to may possibly be present in the sea urchin's egg, and that such materials are not seriously disturbed by a centrifugal force sufficient to separate the visible substances of the egg; but that if these more fundamental substances are displaced, interference with the normal development would be expected. According to this conception vital organization would still be a function of equilibrium and interaction of diverse substances. The logical possibility of such a conception must be admitted, but it avoids none of the difficulties inherent in the usual hypothesis of organization, and seems to me to offer no possibility for explaining the polarity or bilaterality of parts of an ovum or the phenomena of regulation in general.

The existence of polarity and bilaterality in an optically homogeneous medium, and the persistence of both as to orientation under experimental conditions that seriously modify the quantitative relations of the oriented medium in different regions (as, for instance, when the yolk granules are packed closely into the small cell of the two-celled stage of *Chætopterus*) seem to me to argue for a molecular basis of the fundamental principle of vital organization.

Lehmann's observations on fluid crystals offer interesting analogies that are suggestive in the consideration of such fundamental biological problems. They show that the conception of crystallization as applied to vital organization does not meet with some of the difficulties formerly considered inherent in the analogy. Particularly his observations on the so-called myelin forms where innumerable fluid crystals determine the form and behavior of a single aggregate, suggest most interesting biological analogies, and aid to a certain extent in conceiving a doubly heteropolar condition of aggregation such as is presented by the animal egg.[1]

---

[1] It is not of course the author's intention in this communication to attempt to establish the validity of the analogy, but only to point out its possible application to the demonstrated organization of the ground substance of the cytoplasm. A good discussion of the entire analogy of crystallization to morphogenesis is contained in Przibram's article "Kristall-Analogien zur Entwicklungsmechanik der Organismen," *Archiv für Entw'mech.*, Bd. XXII., 1906.

The principle laid down here as to the molecular basis of cytoplasmic organization must apply not only to the stages considered but to all stages of development and to all parts of the organism.

The polarity of the egg and of living parts generally has been compared to magnetic polarity. Thus parts of a magnet exhibit polarity in the same direction as the original whole, and the north pole of the part is as true a north pole as the north pole of the whole magnet ; the same is generally true of polarity in living things : a piece of *Hydra*, for instance, exhibits the same polarity as the entire animal of which it is a part. But push the comparison a little farther and the analogy appears to break down : a piece from the north end of a magnet has no more northness, so to speak, than a piece of the same size from the south end ; on the other hand, an animal piece of an entire egg or an oral piece of an entire animal frequently has considerably more animal or oral properties than a piece from the vegetative or aboral end. The same difficulty of course arises if we use the analogy of crystallization. But it may be that it is due to secondary causes that modify the result without detriment to the validity of either analogy.

In conclusion a few words with reference to the so-called formative stuffs : So far as they are to be identified with the visible substances segregated by the centrifuge, it would appear to be indicated by the experiments that they can play no specific rôle in differentiation, because in centrifuged eggs they may occupy variable positions in the embryo. I believe, however, that the matter requires further investigation. It seems hardly credible that the strictly determinate distribution of these substances in normal ontogeny is meaningless, and that abnormal distribution is a matter of indifference. It is noteworthy that a large proportion of centrifuged eggs die before development has proceeded far, and that there is a distinct tendency towards abnormalities in the surviving eggs. It is natural to correlate these facts with unregulated abnormalities of distribution of granular materials. In any event a more careful study of the facts is needed.

It should be borne in mind that only the factors of polarity and bilaterality, the localizing factors in general, are shown to be functions of the ground substance. The respective rôles of nucleus and granules are undetermined by these experiments; but their importance in the physiology of development is not open to question.

LITERATURE.

Lillie, Frank R.

'06 Observations and Experiments Concerning the Elementary Phenomena of Embryonic Development in Chætopterus. Journ. Exp. Zoöl., III., 1906.

'08 On the Specific Gravity of Constituent Parts of the Egg of Chætopterus and the Effect of Centrifuging on the Polarity of the Egg. Abstract, Science, N. S., Vol. XXVII., June, 1908.

'08 A Contribution Towards an Experimental Analysis of the Karyokinetic Figure. Abstract, Science, N. S., Vol. XXVII., June, 1908.

Lyon, E. P.

'05–6 Some Results of Centrifuging the Eggs of Arbacia. Amer. Journ. Phys., Vol. XV., 1905–1906.

Morgan, T. H., and Lyon, E. P.

'07 The Relation of Substances of the Egg, Separated by a Strong Centrifugal Force, to the Location of the Embryo. Arch. Entw'mech., XXIV., July, 1907.

Morgan, T. H.

'08 The Location of Embryo-forming Regions in the Egg. Science, N. S., August, 1908.

Lehmann, O.

'08 Scheinbar lebende Krystalle, Pseudopodien, Cilien und Muskeln. Biol. Centralblatt, Bd. XXVIII., Aug., 1908.

'04 Flüssige Krystallen. Leipzig, Wilhelm Englemann, 1904.

A NEW NARCOMEDUSA FROM THE NORTH ATLANTIC.[1]

R. P. BIGELOW.

Late in the summer of 1899, the U. S. S. "Fish Hawk" of the Bureau of Fisheries made an expedition from Wood's Hole to the Gulf Stream. Upon her return I was handed a bottle containing a beautiful, clear, colorless medusa of considerable size, and was asked to identify it. The specimen was preserved in formalin and was in excellent condition. A short examination, however, was sufficient to prove it to be an unfamiliar form.

Fearing the deterioration of this unique specimen, I began at once in the Fisheries Laboratory at Woods Hole, as careful a study of its structure as could be made without destroying the specimen. Detailed, measured drawings were made, two of these being reproduced in the present article.

Unfortunately the otoliths were not visible, probably having been destroyed by the formalin, and the specimen appeared to be immature, as gonads were not distinctly marked. These circumstances made the identification difficult, and although the specimen appeared to belong to a new species, I hesitated to describe a new species from this single specimen. It was, therefore, put aside in the hope that another season might furnish material for a more complete diagnosis.

The material hoped for, however, has not appeared, and as my friend, Dr. A. G. Mayer, assures me that in all probability this is a new species of the genus Pegantha, I venture to publish the following description :

PEGANTHA CLARA, sp. nov.

General description. — Bell lenticular, doubly convex, 53 mm. wide, 20 mm. thick, exumbrella smooth, 28 equally spaced bell-lappets alternating with 28 tentacles ; 14 of these tentacles are long, and range between 33 and 56 mm. in length, and they alternate with 14 smaller tentacles ranging in length from 11 to 35 mm. (The specimen in hand has only 27 tentacles, one of the smaller series having failed to develop.) The insertions of the long set of tentacles are farther upward (above the margin) and inward (toward the center) than are the insertions of the short tentacles. The tentacles taper gradually to their pointed tips, and their entodermal cores are composed of a row of chordate cells.

---

[1] Published by permission of Hon. George M. Bowers, U. S. Commissioner of Fisheries.

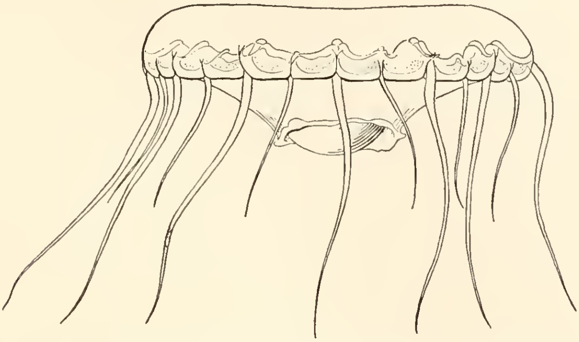


FIG. 1. *Pegantha clara*, side view. The tentacles are represented on one side only. Natural size.

The otolith-clubs appear to have been destroyed by the formalin in which the medusa is preserved; but there are 2–5, usually 3, long, slender, linear, somewhat tortuous, sensory tracts (otoporpæ) which extend from the bell-margin about one half the distance up the exumbrellar side of each lappet.

The velum is simple, annular, and provided with powerful circular muscles. The mouth is a simple annular opening at the thick lenticular center of the subumbrella.

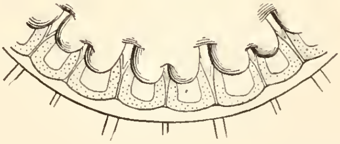


FIG. 2. A portion of the margin of the subumbrella with the velum pressed out flat, and showing gastric pouches, festoon canal, and the transparent bases of the tentacles between them. Natural size.

At the margin of the stomach are 28 simple, unilobular saccules which project downward into the bell-cavity, one in each antimere. These bag-like protrusions thus alternate with the radii of the tentacles, and they contain the genital organs, the specimen being a male. The marginal ring canal, or "festoon canal," is very wide. It extends down the sides of the peronial strand on either side of the insertion of each tentacle, and along the margin of each lappet. It is thus broken up into 28 loops, one in each antimere.

The gelatinous substance of the bell is hyaline. The tentacles, gonads, stomach, and festoon canal are milky — slightly brown in formalin.

The medusa upon which this description is based is probably immature; for one half of its tentacles are of small size, and belong apparently to a set that is in process of development.

Locality. — North Atlantic, U. S. S. "Fish Hawk" Station No. 7068, near the border of the Gulf Stream, off the southern coast of New England.

Vol. XVI. *February, 1909.* *No. 3.*

BIOLOGICAL BULLETIN

AN EXPERIMENTAL STUDY ON THE DEVELOPMENT OF THE VASCULAR AREA OF THE CHICK BLASTODERM.[1]

J. THOS. PATTERSON.

The question regarding the source of the cell-layer in which occurs the formation of vascular tissue of the opaque area of the bird blastoderm has attracted much attention on the part of students of avian embryology. While the solution of this problem has been sought almost entirely through the study of sections, yet this method of attack is met with many difficulties. It is almost impossible to determine by histological means the exact part played by each of the germ layers in the formation of the vascular tissue of the area opaca. It is not my purpose to discuss the extensive literature that has grown up around this problem; but I shall simply state the two views that are commonly held with reference to the origin of the mesoblast, out of which arises the vascular tissue of the area opaca. According to one view it is derived from the germ-wall, by a delamination; according to the other, it is merely a peripheral extension of the primitive streak mesoblast. If the former view be correct, it is obvious that the germ-wall ought to give rise to vascular mesoblast, irrespective of the presence of the primitive streak mesoblast. In other words, if the primitive streak mesoblast were prevented from growing out over, and coming in contact with the germ-wall, the latter ought still to give rise to vascular mesoblast.

Experiment I.—The experimental test for this proposition is a very simple one, and consists in making injuries on an unincubated blastoderm just inside the lateral margins of the area pellucida (Fig. 1, *A*). Such injuries effect a fusion between the ectoderm and entoderm,[1] and thus put up a barrier beyond which the primitive streak mesoblast cannot pass in its peripheral extension.

---

[1] Contributions from the Zoölogical Laboratory of the University of Texas, No. 95.

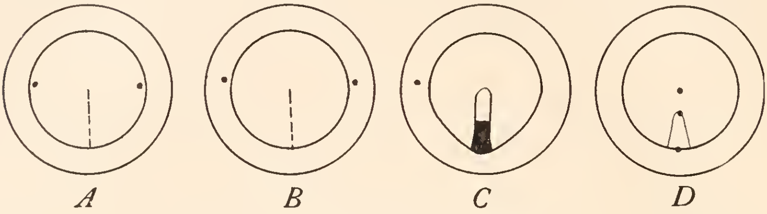


FIG. 1. *A–B* are schemes for operating in Experiments I.–IV. respectively.

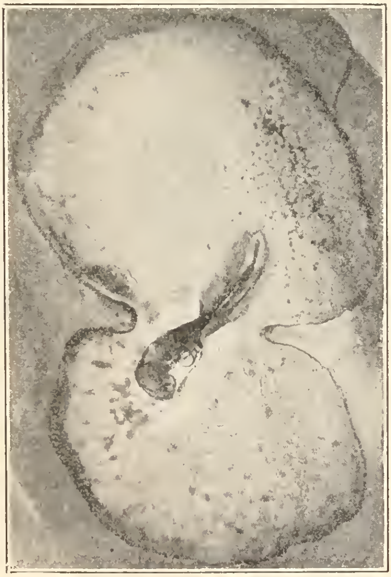
The embryo, as it appears after forty-eight hours of incubation, is shown in Fig. 2. Instead of being circular in outline, as under normal conditions, the vascular area is in the form of a figure eight, the vena terminalis baying in on each side around the obstructing injury-scars. It is easy to see how this result was brought about. The lateral edges of the mesoblast, growing out from the primitive streak, came in contact with the two injuries and at these points the mesoblast was prevented from spreading farther, and so from coming in contact with the germ-wall. There has been also a decided retardation in the spreading of the mesoblast adjacent the injuries. Hence, the transverse diameter of the vascular area, even at the widest points, is only 8 mm., while the longitudinal diameter is 13 mm.

The interesting point brought out by this experiment is made evident when one turns to a study of a section taken across one of the bays (Fig. 3, line *A–B*). The portion of the germ-wall, which lies between the two sections of the vena terminalis, remains undifferentiated (Fig. 4), that is, it has not given rise to vascular mesoblast. It should be noted that it is from this region of the germ-wall that the primitive streak mesoblast has been excluded as a result of the operation. The portion of the germ-wall in the region of the section does not differ histologically from that lying beyond the anterior or posterior limit of the vascular area, yet it is at least twenty-four hours beyond the time when it should have given rise to vascular mesoblast (if the first view stated above be correct). Nor does it give any evidence of so differentiating later, even in experiments in which development has been allowed to go on as long as seventy-two hours. This makes it practically certain that in the absence of the primitive streak mesoblast, the germ-wall does not give rise to vascular mesoblast.

---

[1] The methods employed in this work have already been described in the following papers: Patterson, J. T., "The Order of Appearance of the Anterior Somites in the Chick," BIOL. BULL., Vol. 13; "Gastrulation in the Pigeon's Egg—A Morphological and Experimental Study" (in press), *Journal of Morphology*.

Experiment II.—A less conclusive experiment than the preceding consists in making injuries out in the area opaca (Fig.



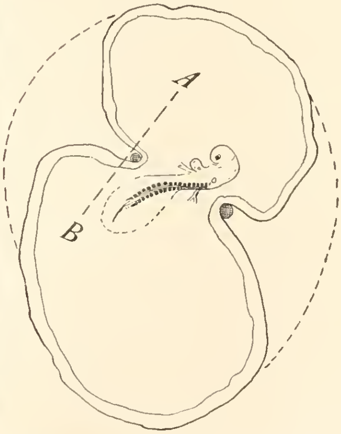


FIG. 2. Photograph of the resulting embryo in Experiment I. The operation was performed on the unincubated blastoderm (see Fig. 1, *A*), and the egg was then incubated for forty-eight hours. The embryo is well developed with twenty-four somites well differentiated. The lateral expansion of the vascular area has been prevented by the two injuries. × 5.8.

FIG. 3. Outline sketch of the embryo shown in Fig. 2. The broken lines at the sides indicate approximately the limits to which the lateral parts of the vena terminalis would have reached had the blastoderm been uninjured. × 4.4.

1, *B*). It gives the same general result, in that the lateral expansion of the vascular area has been retarded (Fig. 5). Sometimes, however, as in the case of the right side of this embryo, the lateral margins of the vena terminalis within the bay grow towards each other and finally fuse, thus leaving the scar of the injury within the margin of the vascular area. In the majority of cases, however, this does not occur.

Experiment III.—Another experimental method of demonstrating that the primitive streak mesoblast is the source of the vascular mesoblast of the area opaca, consists in destroying a given portion of the early primitive streak and then noting the effect on the development of the vascular area. A few of these experiments may be briefly mentioned. If the posterior part of the early primitive streak, together with a small region of the adjacent area opaca,[1] be destroyed, the posterior end of the vascular area is wanting in the later embryo; but the anterior parts of the area are entirely normal. Again, if the anterior tip of the early primitive streak be destroyed, the later embryo shows a deficiency in the anterior part of the vascular area. Finally, if the posterior part only of the primitive streak be destroyed (Fig. 1, *C*), the later embryo lacks the lateral parts of the vascular area which lie opposite the injuries (Fig. 6). In other words, the destruction of any portion of the early primitive streak results in producing a deficiency in the part of the vascular area lying at the level of the injury.

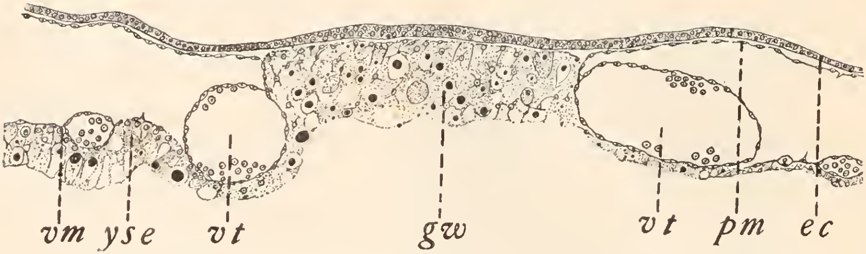


FIG. 4. This is the central part of the section taken in the plane *A–B*, Fig. 3. *vt*, vena terminalis; *ec*, ectoderm; *yse*, yolk-sac entoderm; *pm*, parietal layer of mesoderm; *vm*, vascular layer of mesoderm; *gw*, portion of the germ-wall lying between the two sections of the vena terminalis. Note that it is not differentiated into vascular mesoblast.

---

[1] This is done in order to destroy that part of the primitive streak material which overlaps the area opaca at the posterior border of the area pellucida.

Experiment IV.—The foregoing experiment shows conclusively that the differentiation of one part of the vascular area does not depend on the previous development of another part. It does not answer the question, however, as to whether the development of the vascular area is dependent on the progressive differentiation of the embryo. There are a number of ways (experiments) by which one can prevent the development of the embryo, and at the same time show that the primordium for the entire vascular area is laid down irrespective of the differentiation of the embryo beyond the primitive streak stage. One of the simplest of these is shown in Fig. 1, *D*, and the resulting blastoderm in Fig. 7. Although there is not the slightest trace of the embryo proper present, yet the vascular system of the area opaca

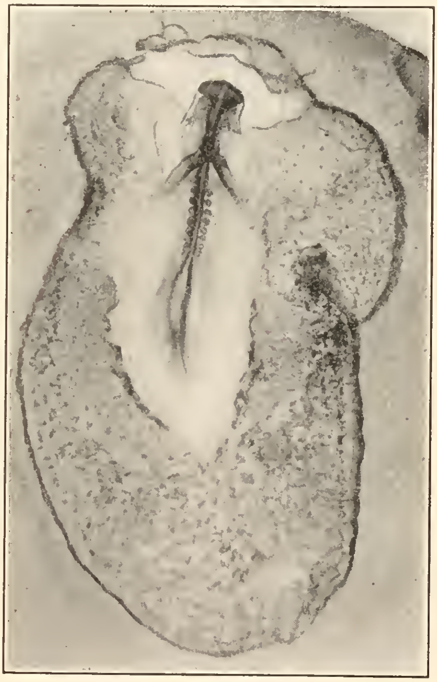


Fig. 5. Photograph of the resulting embryo in Experiment II. The operation was made on the unincubated egg (see Fig. 1, *B*), and the egg was then incubated for forty-nine hours. While development has been greatly delayed, yet the embryo proper is normal, and eight somites are formed. × 12.

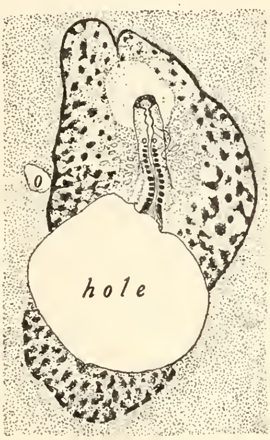
is well laid down, and even the large vessels are seen to pass inwards to the center of the pellucid area. Attempts to develop such blastoderms to the seventy-second hour have failed, from which fact it would seem that after about the forty-eighth hour of incubation the expansion of the vascular area over the yolk is dependent on the propulsion of the newly formed blood-cells through the vessels, that is, on the development of the embryo with its heart.

Discussion.—The results obtained in experiments I. and II. make it practically certain that in the absence of the primitive streak mesoblast the germ-wall does not give rise to vascular mesoblast. Hence, the view that the mesoblast of the area opaca is split off from the germ-wall is doubtless incorrect. There is good evidence, of course, to show that the mesoblast in this area must receive material from the germ-wall. Lillie has pointed out that the mesoderm cells of the area pellucida are void of yolk-granules, while those of the blood-islands "contain yolk-granules of precisely the same character as those of the germ-wall." He states, "therefore, either the blood-islands are derived from the cells of the germ-wall, or cells of the mesoderm growing out over the germ-wall burrow into the latter, engulf yolk-spheres, and reappear in masses as blood-islands."[1] The former of these alternatives is disproved by the result obtained in experiment I.; the latter, therefore, must be regarded as the correct view. In this "feeding" of the mesoderm cells on the germ-wall material during the formation of blood-islands, we doubtless have an explanation for Rückert's[2] contention, viz., that in blood-island formation the primitive streak mesoderm receives elements from the germ-wall; for it is difficult to imagine how it would be possible for the mesoderm cells to take up germ-wall material *en masse* without also including many elements (cells) therein contained. However, the participation of the germ-wall with the primitive streak mesoderm in the development of vascular tissue, must be regarded as secondary (perhaps merely furnishing nutriment), and the power to differentiate blood-islands, therefore, must be located in the primitive streak mesoderm. To attribute such a power to it is not inconsistent with other facts, for it develops blood-islands in the area pellucida, where there is no germ-wall; but for the rapid and abundant development of islands the mesoderm must come in contact with the germ-wall, where it receives an abundance of food-stuffs. If, however, the germ-wall elements actually develop into vascular tissue, they must receive their initiating stimulus from contact with the mesoderm.

---

[1] Lillie, F. R., "The Development of the Chick," New York, 1908, p. 89.

[2] Rückert, J., "Entwickelung der extra-embryonalen Gefässe der Vögel," "Handbuch der vergl. w. exp. Entw.—lehre der Wirbelthiere," Bd. I., T. 1, 1906.



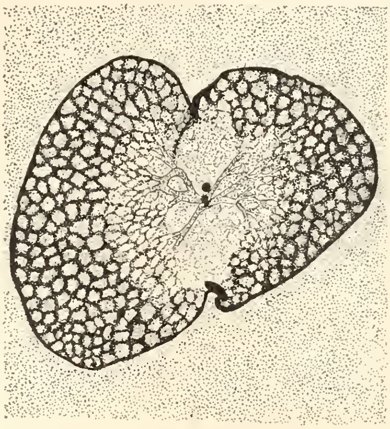


Fig. 6. The resulting embryo in Experiment III. (see Fig. 1, *C*). The operation was made after nine hours of incubation, and the egg was then incubated for thirty-four hours. The region destroyed by the operation is represented by a large hole, opposite to which the vascular area is wanting. Posterior to the hole is a small area of vascular tissue, which doubtless has been formed from the part of the primitive streak material overlapping the area opaca at the posterior border of the area pellucida. The anterior part of the vascular area is slightly asymmetrical, owing to the retardation of the spreading of the mesoblast on the left side, caused by the injury made on this side in the area opaca. × 5.4.

Fig. 7. The resulting embryo in Experiment IV. (see Fig. 1, *D*). The operation was made after about seven hours of incubation, and the egg was then incubated for fifty hours. The embryo is entirely wanting, but the vascular area is well developed, except at the posterior end where its spreading has been retarded by the most posterior injury. The lateral parts of the vascular area consist of a net-work of vessels, and in the region of the pellucid area large vessels pass in towards the center. No heart, however, is developed. × 5.8.

In addition to supporting the above contention, the results obtained in experiment III. also do away with the necessity for assuming that the vascular area must start as an "anlage" at the posterior border of the area pellucida. There is no doubt but that the first blood-islands appear here, but this is explained by the fact that the primitive streak mesoderm (from primitive plate) is from the first in close proximity to, if not in direct contact with, the germ-wall at this point. The progressive differentiation of the blood-islands from here anteriorly around the inner edge of the area opaca is only an index to the progress made in the expansion of the primitive streak mesoderm.

THE REACTIONS OF DIDINIUM NASUTUM (STEIN) WITH SPECIAL REFERENCE TO THE FEEDING HABITS AND THE FUNCTION OF TRICHOCYSTS.[1]

S. O. MAST.

Outline.

Introduction 91
Description 92
Locomotion and General Reaction 92
Variation in Form 94
Variation in Size, Fission and Encystment 95
Feeding Habits 96
Function of Trichocysts 103
Mechanics of Swallowing 108
Amount and Kind of Food 112
Choice of Food 113
Summary 117

Didinium nasutum has been known for more than a century. It was described by O. F. Müller in 1786 under the name of *Vorticella nasuta*. In 1859 Stein again described this creature and gave it the now generally accepted name, *Didinium nasutum*. Balbiani (1873), however, seems to have been the first to study and record its peculiar feeding habits and his account, although wrong in many respects, had been generally accepted until the paper of Thon (1905) appeared. This paper, however, as the title indicates, deals largely with the structure of the animal and contains only a brief account of the feeding habits. Since this account as well as that of Balbiani seems questionable, I decided to study the reactions of this interesting organism more in detail than had previously been done.

In order to present the results of this study[2] clearly, it will be necessary to give a brief description of the organism.

---

[1] From the Laboratory of Experimental Zoölogy, Johns Hopkins University.

[2] The experiments forming the basis of this paper were performed in the Zoölogical Laboratory of Johns Hopkins University, in connection with other work, an account of which will be published later. I am much indebted to the university for exceptional facilities put at my command and for financial aid extended to me as Johnston Scholar.

Description.

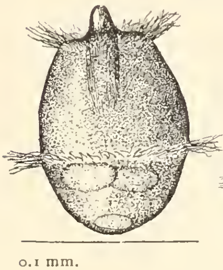
Didinium nasutum is a ciliate protozoön considerably smaller than *Paramecium* (Figs. 1–4). It can scarcely be seen with the naked eye. It is approximately ellipsoidal in form and grayish in color. One end, the anterior, is slightly flattened. From the middle of this end there arises a conical protuberance at the apex of which the mouth is situated. Two distinct bands of cilia encircle the body, one near the middle and the other very near the anterior end. Near the posterior end there is a contractile vacuole and near the central part of the body a relatively large nucleus, which is frequently bent upon itself something like a figure eight. The conical projection appears fibrous. It contains numerous rod-like structures, many of which extend almost to the center of the body. These rod-like structures can be isolated by crushing the animal. They are insoluble in water and appear to be quite rigid. Some of these structures are imbedded in the protoplasm near the surface and appear to serve as support for the conical protuberance, especially during the process of swallowing. The rest are centrally located. Taken together, they form a cylindrical structure referred to by Thon as "mittlerer Strang." *Didinium* seizes and holds its prey by means of this structure. I shall therefore call it the seizing organ.

Didinium nasutum is occasionally found in cultures containing *Paramecia;* it usually appears after most of the *Paramecia* have died out, but is seldom very abundant in such cultures. There is, however, little difficulty in obtaining large numbers by judicious feeding on *Paramecia*, which it captures and swallows entire.

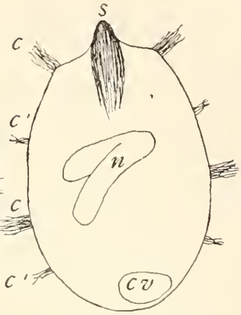
Locomotion.

Didinium is a very active creature. One seldom finds it at rest. It swims about rapidly, constantly rotating clockwise on its longitudinal axis and at the same time swerving toward a given side. This causes it to proceed on a spiral course which is usually not over 2 mm. wide. When the organism comes in contact with anything, it ordinarily suddenly reverses the stroke of the cilia, backs a short distance, turns toward one side, and then proceeds on a new course at an angle with the old. That is, it responds with an avoiding reaction similar to that discovered by Jennings in *Paramecium, Oxytricha,* etc. One of the striking peculiarities about this creature is the fact that in responding with the avoiding reaction, it always turns toward the same side. One

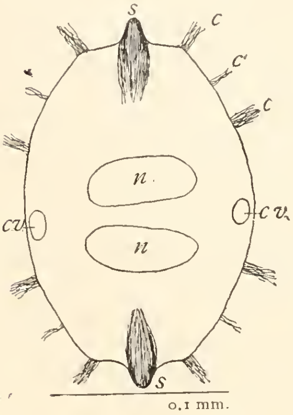
2

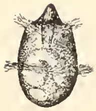


3

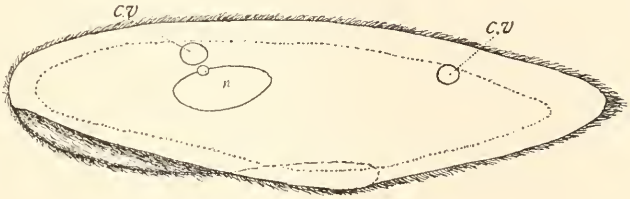


4





1



5

Fig. 1.[1] *Didinium nasutum,* one of the smallest specimens found. Such specimens result when repeated fission takes place in the absence of food.

Fig. 2. One of the largest not in the process of division.

Fig. 3. One of the largest specimens just beginning the process of fission as indicated by the additional bands of cilia, *c'*; *c,* primary bands of cilia; *n,* nucleus; *c.v.,* contractile vacuole; *s,* seizing organ; 0.1 mm., projected scale.

Fig. 4. One of the largest monsters seen. The narrow bands of cilia, *c'*, indicate the beginning of fission.

Fig. 5. A typical specimen selected from a race of exceptionally large *Paramecia.* Compare with *Paramecium* in Fig. 6.

---

[1] All the figures accompanying this paper were outlined with the camera from specimens killed suddenly with Worcester's fluid and preserved, after washing with water, by adding ten per cent. glycerine solution and allowing it to concentrate by slow evaporation. No change in size or form could be detected during this process.

would hardly expect this in an organism so nearly radially symmetrical that it is impossible to see any fixed structural differences between the various sides.

That these animals do, however, always turn toward the same side was ascertained in two ways: (1) By withdrawing the water from under the cover-glass until they could no longer swim freely; and (2) by adding Chinese ink to a thin solution of quinced jelly and studying their reactions in this. Under the cover-glass in only a very thin layer of water they could of course turn only in two directions and thus it was very easy to see that they always turned toward the same side under these conditions. When free to swim in all directions, it was much more difficult to follow their movements precisely. In the jelly solution, however, the avoiding reactions are so frequent and the rate of motion is so much reduced that by selecting a specimen to the surface of which particles of ink adhered in such a way as to differentiate the sides, it could be definitely seen that they always turn toward the same side.

By direct observations on the movement of the cilia during the process of turning, and by studying the currents produced, it could be definitely seen that the cilia in the anterior band along the edge toward which the animal turns, strike forward toward the mouth, while the rest in this band and all those in the posterior band strike backward. The fact that some of the cilia in the anterior band strike in one direction at the same time that others in the same band strike in the opposite direction and that this occurs only when the creature is turning, shows an interesting adaptive differentiation in the function of cilia.

In this organism there is no evidence of differential response to localized stimulation. *Didinium* always turns toward the same side, no matter which part of the anterior end comes in contact with an object, the conical projection or various portions of the edge.

Variation in Form.

Not infrequently one finds specimens of *Didinium* which apparently consist of two or three individuals fused together at the posterior ends. These specimens are about two or three times normal size, depending upon the number fused, and all the different parts of normal specimens are duplicated, the bands of cilia, contractile vacuoles, nuclei, seizing organs, etc. When two individuals are fused together, the anterior ends usually point in opposite directions, as represented in Fig. 4, but a few specimens were found in which they pointed in the same direction. Only one specimen composed of three individuals was found. In this the individuals were symmetrically arranged, forming a structure something like a three-pointed star. All these abnormal creatures appear to swim equally well with any one of the anterior ends ahead. They feed just as normal animals do, but I have never found any that reproduced, although at various times isolated specimens were under observation for several days. One specimen, however, was found with two additional bands of cilia indicating that the process of fission had begun (see Fig. 4).

I am unable to account for the origin of these monsters. One might naturally assume it to be a case of imperfect fission, but this is improbable, for in fission an anterior end and a posterior end form at the plane of separation, while in these creatures posterior ends are always in contact.

Variation in Size, Fission and Encystment.

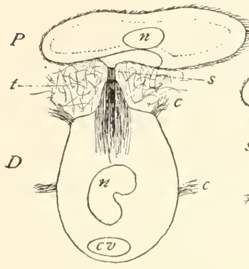
The results of the study of the feeding habits of *Didinium* depend largely upon the relations in size between the *Didinia* under observation that the *Paramecia* upon which they are feeding. Both of these forms vary much more in size than is ordinarily supposed. The smallest *Didinium* obtained (see Fig. 1) measured 0.058 mm. in length and 0.034 mm. in width. The largest normal specimens (Fig. 2) with but two bands of cilia, that is, specimens which were not about to divide, measured 0.130 mm. in length and 0.085 mm. in width. They were therefore more than twice as long and nearly three times as wide as the smaller specimens, which means that the larger are about six times as great as the smaller. Some of the specimens (Fig. 3) about to divide measured 0.166 mm. by 0.101 mm. and one of the largest abnormal specimens (Fig. 4) was 0.207 mm. in length and 0.126 mm. in width, nearly four times as long and four times as wide as the smallest specimens, and about fourteen times as large. *Paramecia* vary nearly if not quite as much in size as *Didinium*. One of the smallest found in the cultures worked with measured 0.117 mm. in length and 0.034 mm. in width and one of the largest was 0.339 mm. long and 0.095 mm. wide, and there were many others of similar size, both small and large, in the cultures. A clear conception of variation in size can be gained by referring to the various figures presented in this article.

The amount of food available is one of the chief factors in regulating the size of *Didinium*. In the absence of food they do not immediately encyst as Thon (p. 293) says, but continue to divide by fission, becoming smaller and smaller after each division. On May 1 at 10:00 A. M. five exceptionally large specimens were taken from a culture and put into a solution without food. At 4:00 P. M. there were six present, one of which was a double monster. May 3 at 9:00 A. M. eleven; May 4, 9:00 A. M., eighteen; May 5, 9:00 A. M., twenty-three; May 6, 9:00 A. M., twenty-six; May 7, 9:00 A. M., twenty. After this the number decreased. I am not positive that they encysted. The monster did not divide at all, consequently twenty-five specimens were produced from four in the total absence of food. One would therefore expect the individuals of the final generation to be much smaller than those of the original. They were in fact about one sixth as large.

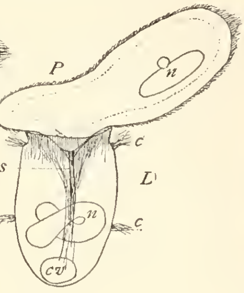
These experiments show that size has little if any influence on fission and that absence of food does not primarily cause *Didinium* to encyst. Neither does the presence of food cause these organisms to come out of the cysts. This was clearly shown by adding *Paramecia* to solutions containing encysted *Didinia*. If only a small amount of liquid was transferred with the *Paramecia*, the *Didinia* seldom came out of the cysts; on the other hand, they were induced to come out on several occasions by merely adding water. This seems to indicate that the chemical contents of the water in which the *Didinia* live has much to do with encystment. The relative importance of the various factors involved in this process is, however, a subject for future investigation.

Feeding Habits.

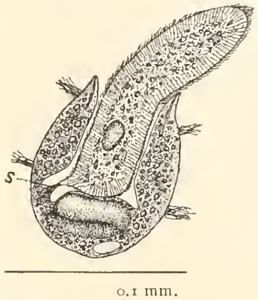
If a number of *Didinia* are studied under a lens, they appear to be darting about in wildest confusion, sharply turning to the right and left, up and down, and frequently running into each other. This is especially true if they have been without food for a few days. In thus rapidly swimming about in every direction, they cover a large space in a short time and come in contact with everything that may happen to be in this space. If a *Didinium* chances to swim against a *Paramecium* in these aimless maneuvers, the two frequently adhere to each other and one soon finds a tangle of fine filaments all about the scene. The whole mass, filaments and all, now moves about as one, but the *Paramecium* disappears in the course of from one to three minutes, having been swallowed by the *Didinium*.



6



7



8

FIG. 6. A large *Didinium* immediately after having seized a small *Paramecium*. *D, Didinium; P, Paramecium; n. nucleus; c,* bands of cilia; *c.v.*, contractile vacuole; *s*, seizing organ, showing small granules seen at the point of contact between the seizing organ and the surface of the *Paramecium; t,* discharged trichocysts; 0.1 mm., projected scale.

FIG. 7. A specimen showing the seizing organ withdrawn and the beginning of the process of swallowing.

FIG. 8. A specimen in which the seizing organ, *S*, could be clearly seen attached to the prey after it had been nearly half swallowed. This shows that the seizing organ travels to the posterior end of the body during the process of swallowing and in so doing no doubt draws in the prey.

Balbiani[1] (1873, p. 363) describes this process as follows: "If while swiftly turning in the water, the *Didinium* happens into the neighborhood of an animalculum, say a *Paramecium*, which it is going to capture, it begins by casting at it a quantity of bacillary corpuscules which constitute its pharyngeal armature. The *Paramecium* immediately stops swimming and shows no other sign of vitality than feebly to beat the water with its vibratile cilia; on every side of it the darts lie scattered that were used to strike it. Its enemy then approaches and quickly thrusts forth from its mouth an organ shaped like a tongue, relatively long and resembling a transparent cylindrical rod; the free extended extremity of this rod it fastens upon some part of the *Paramecium's* body. The latter is then gradually brought near by the recession of this tongue-shaped organ towards the buccal aperture of the *Didinium,* which opens wide, assuming the shape of a vast funnel in which the prey is swallowed up."

---

[1] Translation quoted from "Psychic Life of Micro-organisms," by Binet. Published by the Open Court Publishing Company, Chicago, Illinois, 1889, p. 54.

Thon (1905, p. 294) maintains that the *Paramecia* are not killed by trichocysts but by the seizing organ: "Das erste was wir bei der Verfolgung des *Didinium* manövers konstatieren können, ist, dass die Abtötung des *Paramaecium* nicht durch 'Trichocysten' sondern durch den mittleren Strang bewirkt wird. Das lebhaft rotierende *Didinium* tastet mit dem Rande des Mund öffnung an den herumschwimmenden *Paramaecium* umher. Plötzlich schiesst es durch eine energische Kontraktion des Pharynx mit wunderbarer Geschwindigkeit den mittleren Strang hervor, welcher dann aus der Mund öffnung wie ein schlanker, heller Cylinder heraus ragt und manchmal 2/3 Körperlänge erreicht. Dieser Strang wird nun in den Paramaeciumkörper sehr fest eingebohrt und führt den plötzlichen Tod des *Paramaecium* herbei."

Thon is undoubtedly correct in his statement that the *Paramecia* are not killed by trichocysts. As a matter of fact, *Didinium* discharges none. The mass of trichocysts seen after a *Paramecium* is captured by a *Didinium* all come from the *Paramecium,* and not from the *Didinium* as Balbiani and Maupas assumed. This can be readily demonstrated by feeding the *Didinia* organisms which have no trichocysts, *e. g., Colpoda* or *Colpidium.* I have repeatedly seen these protozoa captured and swallowed by *Didinia* but never saw trichocysts discharged during the process. Are the *Paramecia* really poisoned by the seizing organ and suddenly killed as Thon states, and is this organ actually thrust out to seize the prey?

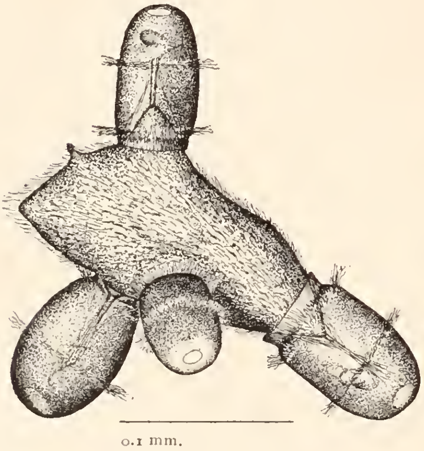
Didinia are powerful swimmers. If one becomes fastened to a *Paramecium* even twice its own size, then the *Paramecium* at once begins to rotate in harmony with the *Didinium,* is carried off and on casual observation it appears to be dead or at least thoroughly paralyzed. If, however, the *Paramecium* is very much larger than the *Didinium* the result is quite different.

During the first part of my work on this subject, I had a race of *Paramecia,* none of which were much more than twice as large as the *Didinia.* After studying the food reactions with these specimens and seeing many *Paramecia* captured and swallowed without any apparent struggle and several, which escaped, die immediately, I was of the opinion that Thon (1905, p. 296) is correct in his statement that the *Paramecia* are poisoned and suddenly killed.

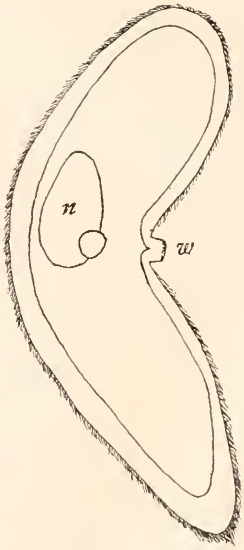
Later, however, there appeared in one of the cultures a race of *Paramecia* which were several times as large as those previously worked on (see Fig. 6, p. 97), and by selecting *Didinia* which had been without food for a few days, it was a simple matter to find specimens not more than one sixth to one tenth as large as these *Paramecia.* These minute *Didinia* attack the huge *Paramecia* as vigorously as they do the smaller ones. In such an attack, however, the scene of action and the result are quite different from that described above. The *Paramecium* attacked does not become quiet, it makes a vigorous struggle and frequently succeeds in throwing off the enemy by breaking the attachment of the seizing organ. I shall discuss the significance of this process later (p. 103). The *Paramecium* thus almost invariably escapes after the first attack and I have frequently seen two and sometimes three *Didinia* successively thrown off before one finally remained attached. And even after one does succeed in this, it merely momentarily checks the movement of the prey. There is no indication of paralysis. The *Paramecium* attacked continues to swim about, carrying the *Didinium* with it. A large specimen studied was thus seen to swim about with a small *Didinium* fastened to its sides for over four minutes, when it was attacked by a second individual, badly wounded and killed. The first individual had swallowed about one fifth of the *Paramecium* before it died, and the second had drawn in a small portion. Both then continued the swallowing process until they had surrounded a large proportion of the body, when the ectosarc gave way and the mouths of the two creatures closed after quite a considerable portion of the substance already swallowed had flowed out.

When the *Didinia* are numerous, several usually attack an animal in rapid succession and it is very soon brought down with a number of these voracious hunters firmly fastened to various portions of the body, as represented in Fig. 9.

Paramecia that escape after having been attacked once or twice very frequently recover. On June 9, at 3:30 P. M., eight such specimens were isolated and put into two watch glasses in a damp chamber. All the specimens were badly deformed, owing to the violent contractions of the surface surrounding the wound. Three died shortly after they were isolated, leaving two living specimens in one watch glass and three in the other. At 10:00



9



10

Fig. 9. A large *Paramecium* attacked by four small *Didinia*. Under such conditions the *Paramecium* is usually torn in pieces and each *Didinium* gets a portion. Sometimes however one *Didinium* gets the entire *Paramecium*, forcing the others off during the process of swallowing.

Fig. 10. A large *Paramecium* which escaped after having been attacked by a *Didinium* showing the wound, *w*, the absence of cilia around the wound, and a marked flexure in the animal, the result of contraction of the ectosarc.

A. M., June 10, there were three in each watch glass. One of the animals had therefore divided. All appeared nearly normal in form, the bulges and depressions due to the wounds having almost disappeared. At 3:00 P. M., June 11, all in the glass that originally had three were dead, but each of those in the other had divided so that there were now six in the dish that originally had two.

All of these observations show very clearly that *Paramecium* is not immediately paralyzed by *Didinium*, as is generally supposed, and that if there is any poison injected by the seizing organ, it is very mild. There are also other organisms which show no indication of immediate paralysis after an attack by this ciliate. Two of these, *Colpoda* and *Frontonia,* were studied in detail. These protoza appear to have an ectosarc which is not as tough as that of *Paramecium,* so that after the seizing organ is fastened to it and the *Didinium* suddenly reverses its directions of motion and jerks back, as it usually does when it swims against an object, the ectosarc frequently gives way and the enemy swims off with a small mass of protoplasm attached to the seizing organ. Sometimes, however, especially in the case of *Colpoda,* the ectosarc does not break until the *Didinium* has drawn the victim part way in and the mouth begins to close. Thus I have seen specimens of *Colpoda* escape, and recover, after having had various portions, even as much as one third of the body, torn away. And in case these creatures do not escape but are swallowed, the cilia can be seen to beat as long as any part projects.

Frontonia is one of the largest of the protozoa. It is much larger than *Paramecium,* but it is nevertheless, vigorously attacked by *Didinium.* The seizing organ readily sticks to the surface of this creature, but the ectosarc almost always gives way as soon as the *Didinium* jerks back after striking it. This is no doubt due, in part at least, to the relatively great momentum of this animal. It takes a much greater force to move *Frontonia* than it does to move *Colpoda,* so that there is a much greater strain on the ectosarc of the former than there is on that of the latter when a *Didinium* suddenly reverses the direction of motion after the seizing organ is attached. Thus the creature attacked escapes, leaving a portion of the ectosarc with the enemy, which the latter proceeds to swallow.

I am not certain as to how soon *Didinium* is able to make a second attack, but from all appearances it can do so in the course of a few minutes. On May 1, a specimen of *Frontonia leucas* (?) was put into a drop of water on a slide containing numerous *Didinia* which had not been fed during the preceding two days. This specimen was attacked 58 times before it finally died, 40.5 minutes after it was put in. A piece was taken out of the creature at every attack. The wounds thus made immediately closed and the animal swam about as though nothing had happened, but it of course became smaller and smaller, until when it finally burst and died there remained but a minute mass of protoplasm nearly spherical in form, with a diameter nor more than one tenth as great as the original length of the specimen. Four other individuals studied the same day were killed in a much shorter time. They lived after the first attack respectively 6, 7, 10 and 12 minutes. But later, June 9, a specimen lived for nearly two hours after the first attack, and was seized literally hundreds of times by small *Didinia* during this period.

I am well aware that the results of all these observations do not prove conclusively that *Didinium* does not poison its prey, for it may be said that the very fact that a piece remains attached to the seizing organ every time that an animal which has been attacked escapes, saves the animal from being poisoned by preventing any toxic substances from entering the remaining portion of the body. This, however, does not apply to the case where the victim remains alive for some time with *Didinium* fastened to it. As stated above, one specimen lived more than four minutes under such conditions.

Let us now briefly consider the evidence in favor of the view that noxious fluid is used by *Didinium* in capturing its food. This view has its foundation in the apparent sudden paralysis and death of the organism captured, described by Balbiani, Maupas and others. Thon, however, presents other evidence supporting this view. He writes as follows (1905, p. 296): "Dass das Plasma des Stranges auf den Körper der Beute ätzend einwirkt, sehen wir wie an lebenden Tieren so auch besonders an den nach Mallory behandelten Präparaten. Sobald dem *Paramaecium* die Wunde verursacht wird, fangen in der nächsten Umgebung sich zahlreiche Vacuolen zu bilden an, die manchmal eine ansehnliche grösse ereichen und das Plasma quillt hervor." There are then two sources of evidence in favor of the view that noxious fluid is injected by *Didinium* in capturing its prey, the apparent sudden death of the prey and the vacuolation in the neighborhood of the wound made by the seizing organ. We have shown conclusively that paralysis is merely apparent, that organisms are not as suddenly killed as they appear to be, and that they frequently recover if they escape after being wounded. The vacuolation can be conceived to be due to physical effects quite as well as to toxic effects. At any rate it is well known that a physical injury in *Paramecium* does cause vaculation and that these organisms are often killed by slight abrasions. It is almost impossible to obtain specimens which have recovered after having a portion of the body cut off.

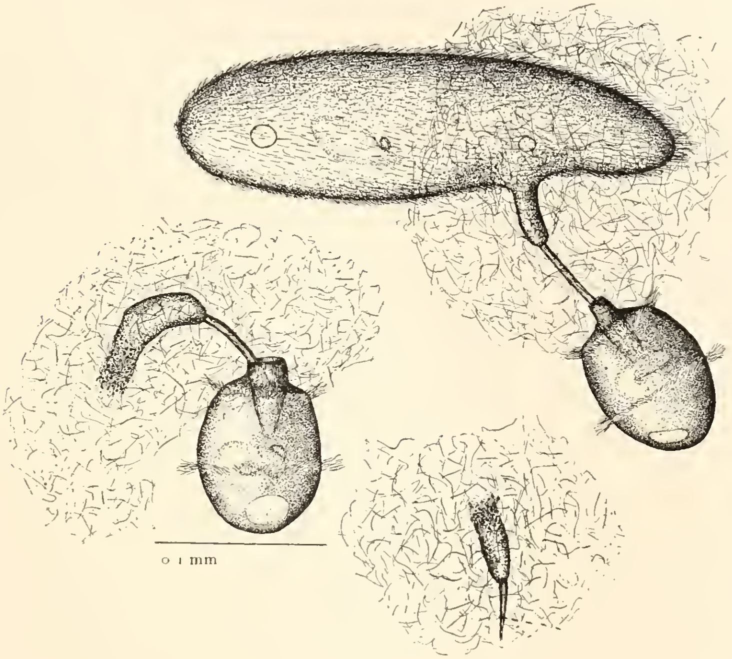
Let us now proceed to the second question stated above. Does *Didinium* thrust out the seizing organ in capturing its prey? This question I shall consider in connection with the function of trichocysts.

Function of the Trichocysts.

If a large number of *Didinia* which have been without food for a few days are thrown into a solution containing numerous *Paramecia,* many of them will begin to feed at once. If they are now suddenly killed, it is not difficult to find specimens attached to *Paramecia* with the seizing organ extended. If the *Paramecia* are relatively small, it will be found to be extended only a short distance, but if they are relatively large, one frequently finds it extended to a distance nearly equal to the length of the body of the *Didinia* as represented in Fig. 11. Balbiani and Thon also represent this organ thus extended. Both of these authors say that it is thrust out. Thon (1905, p. 294) in describing the capture of *Paramecia* says it is forced out by violent contraction with the rapidity of lightning and later (p. 295), in referring to *Didinium* feeding on flagellates, he writes: "Da können wir sehen dass ein *Didinium* in kurzer Frist einiger Minuten eine grosse Zahl von Flagellaten, welche nicht naher bestimmbar ist, einfängt. Blitzschnell wird der Strang hervorgestülpt, mit derselben Geschwindigkeit verschwindet der Flagellat im Innern des Feindes." If this organ was actually shot out with lightning speed, as Thon says, it is difficult to see how one could be certain of just what did happen.

I have studied the maneuvers of *Didinium* in capturing its prey, many times with the greatest care, and was never able to see any indication of such a thrusting out of the seizing organ. What actually happens is this. The *Didinia*, especially when hungry, swim about very rapidly. In such specimens the seizing organ appears to be entirely withdrawn; it cannot be seen to project beyond the surrounding surface in the least. When one of these rapidly moving creatures chances to swim against a *Paramecium*, it strikes with such force that the *Paramecium* can clearly be seen to be pushed out of its course. This is particularly marked if the prey is small. There is no doubt but that the distal end of the oral projection comes in close contact with the surface. This I have actually observed many times. As soon as the *Didinium* strikes an object, *e. g.*, a *Paramecium*, it usually suddenly stops rotating and at the same time reverses its direction of motion. One can never tell whether the seizing organ is fastened or not until this reversion takes place. It very frequently happens that the organ does not become attached during this process and then the *Didinium*, of course, fails to capture its prey. Just why the seizing organ sticks to a *Paramecium* some times and not at others I am unable to explain. All that can be seen in preparations which show the organ fastened is small dots at the point of fusion represented in Fig. 6. It may be that sometimes this organ is entirely withdrawn so that when the hunter strikes a *Paramecium* it does not actually come in contact with the surface.

If the seizing organ becomes fastened, either the fusion with the surface or the pull exerted by the jerking back of the *Didinium* immediately after the fusion produces some sort of injury, for the *Paramecium* at once responds by discharging a great number of trichocysts from the ectosarc for some distance around the point of injury, Fig. 11. As soon as the trichocysts come in contact with the water they form a mass which appears to have a jelly-like consistency; and the increase of this mass due to the extension of the trichocysts forces the two creatures apart, but the attachment between them is not at once broken. The seizing organ draws a portion of the ectosarc out, forming a more or less marked protuberance and it is itself drawn out at the same time. If the *Didinium* is large and the *Paramecium* small, the discharge of the trichocysts has little effect and one sees but a small protuberance and only a slight extension of the



12 13 11

Fig. 11. A large *Paramecium* immediately after having been attacked by a small *Didinium*. The discharge of the trichocysts has mechanically forced the *Didinium* back, drawing the seizing organ out and producing a marked protuberance on the surface of the *Paramecium*.

Fig. 12. A *Didinium* entangled in a mass of trichocysts after the *Paramecium* attacked has escaped by tearing off the protoplasmic protuberance.

Fig. 13. A portion of the seizing organ, *S*, left attached to the protoplasmic protuberance after the *Didinium* has escaped. The *Didinium* has become free by twisting off the seizing organ.

seizing organ, if any; but if the *Didinium* is small and the *Paramecium* large, this organ is frequently drawn out nearly the length of the body, as stated above, and the protuberance of ectosarc becomes so much extended that it breaks and the *Paramecium* swims away, leaving the *Didinium* attached to the bit of ectosarc imbedded in the mass of trichocysts (Fig. 12). It is remarkable what a firm jelly-like consistency this mass has, and how securely the bit of ectosarc pulled out of the *Paramecium* is imbedded in it. The *Didinium* thus fastened to the mass of trichocysts at first appears to be hopelessly entangled but it soon begins to loosen itself by violently jerking backward and darting forward. After it has thus become partly free it begins to rotate on the longitudinal axis and soon twists the seizing organ off at a point in the body some distance from the surface and swims away leaving this important organ in the tangle of trichocysts as represented in Fig. 13. Sometimes the trichocysts give way and the hunter escapes with the bit of protoplasm, which it then swallows. In one such instance, however, which came under my observation, the *Didinium* turned suddenly immediately after it became free, and swam violently against the slide on which it was mounted. The bit of protoplasm adhered to this so firmly that it held the creature fast and it escaped only after twisting the seizing organ off. This illustrates in a striking way one of the properties of bare living protoplasm. I am not positive as to what becomes of these specimens without a seizing organ. They appear normal in every respect and it is altogether likely that the missing organ is regenerated in course of time. There is no evidence that the trichocysts have a toxic effect on the *Didinia,* or that they injure them in any way. They appear to function merely by mechanically separating the prey from its enemy. There are, however, two other factors which assist in this separation: (1) the violent struggle of the *Paramecium* after it is attacked, and (2) the sudden reversion in the direction of motion of the *Didinium*. That these two factors, however, are insufficient in themselves to break the connection or even to account for the extreme extension of the seizing organ and the large protuberance in the ectosarc is evident from the fact that when a *Didinium* attacks a *Paramecium* on a surface where the trichocysts have already been discharged owing to a previous attack, the connection is never broken; the protuberance is insignificant and the seizing organ is only slightly extended if at all. Thus a second hunter frequently succeeds in remaining attached to its prey after the first has been thrown off.

In *Nassula,* a comparatively large ciliate, the trichocysts are of such a nature and so abundant that I have never seen one captured by *Didinium,* although they are vigorously and freely attacked.

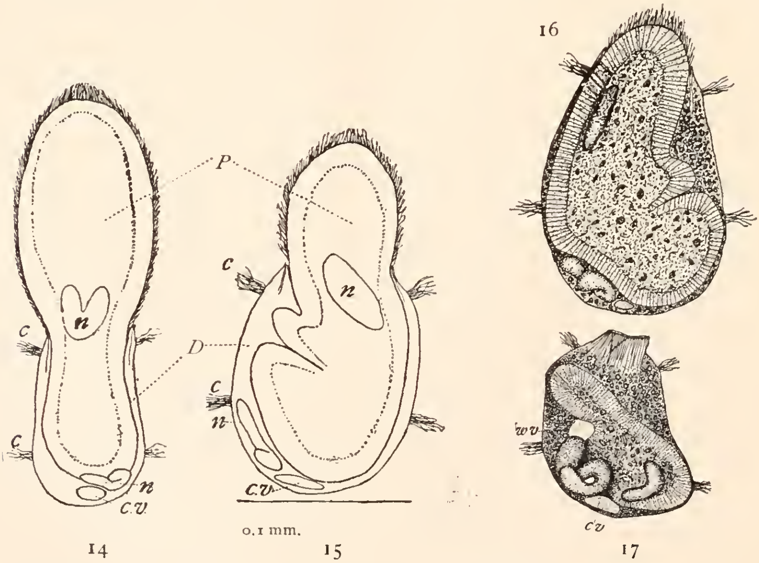
This is, as far as I know, the first instance in which a defensive function of the trichocysts has been actually demonstrated, although it has generally been assumed to be both offensive and defensive. Calkins (1901, p. 50) after referring to the offensive use of these structures in *Didinium,* writes as follows: "This process is strikingly illustrated by the ciliate *Actinobolus radians,* which combines the selection of food with the offensive use of trichocysts. This remarkable organism possesses a coating of cilia and protractile tentacles, which may be elongated to a length equal to three times the body-diameter, or withdrawn completely into the body. The ends of the tentacles are loaded with trichocysts (Entz, 1883). When at rest, the mouth is directed downward, and the tentacles are stretched out in all directions, forming a minute forest of plasmic processes, amongst which smaller ciliates, such as *Urocentrum, Gastrostyla,* etc., or flagellates of all kinds may become entangled without injury to themselves and without disturbing the *Actinobolus* or drawing out the fatal darts. When, however, an *Halteria grandinella,* with its quick and jerky movements, approaches the spot, the carnivore is not so peaceful. The trichocysts are discharged with unerring aim, and the *Halteria* whirls around in a vigorous, but vain, effort to escape, then becomes quiet, with cilia outstretched, perfectly paralyzed." In another section of his book (p. 175), however, referring to the trichocysts in general, Calkins says: "Their function is purely conjectural, although it is generally supposed that they serve as defensive weapons."

Jennings also thinks that we are uncertain as to their function. He writes (1906, p. 90): "They are usually supposed to be weapons of defence. If a *Paramecium* is seized by an animal which is attempting to prey upon it, the trichocysts will of course be discharged from the injured region. But whether they really serve for defence seems questionable. Certainly the infusorian *Didinium*, which is the chief enemy of *Paramecium*, is not hindered in the least from seizing and devouring the animal by the discharge of trichocysts. It is possible that the discharge is really an expression of injury—a purely secondary, even pathological, phenomenon, like the formation of vesicles on the surface of an injured specimen."

It would be of the greatest interest to study the feeding habits of *Actinobolus*, with special reference to the function of trichocysts, in the light of our present knowledge of their function in *Paramecium*.

Mechanics of Swallowing.

It is well known that *Didinia* can capture and swallow organisms which are relatively enormous in size. But it is difficult to obtain the maximum differences in size between the enemy and the victim, for both swim so rapidly that it is impossible to

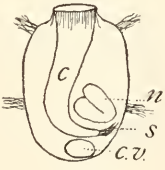


FIGS. 14, 15, 16 and 17. Different stages in the process of the swallowing of large *Paramecia* by small *Didinia*. See text. *D*, *Didinium*; *P*, *Paramecium*; *n*, nucleus; *c.v.*, contractile vacuole; *w.v.*, water vacuole.

measure them with any degree of accuracy while alive. and if they are killed before the swallowing process is complete, one can never be certain that it could have been accomplished had they not been killed. The simplest method seems to be, to fix a large number of specimens immediately after they have been feeding, then select the largest and calculate the relative size of the organism swallowed. This was done and some specimens were found in which the substance of the *Paramecium* swallowed occupied approximately ten times as much space as the substance of the *Didinium*. In such specimens the *Didinium* forms a mere film over the *Paramecium,* as represented in Fig. 16. If other animals could engulf objects relatively as large as this hunter ciliate can, a common garter snake could readily swallow a rabbit, a large house cat a sheep, and a lion or a human being a full grown ox.

What are some of the factors involved in the process of swallowing objects of such extraordinary size?

Didinia attack their prey at any point with which they chance to come in contact, and appear to be able to swallow them equally well no matter whether they become attached to the sides or the ends. If a *Didinium* succeeds in remaining attached to its prey after it has made an attack, it at once begins to draw in the seizing organ. This it can do, no matter how far the organ has been extended by the discharge of trichocysts and other factors. The captured prey is thus drawn to the mouth which gradually opens and surrounds it, as the seizing organ travels toward the posterior end of the animal. The whole process is



18

FIG. 18. A *Didinium* showing a canal, *c,* formed by the invagination due to the movement of the seizing organ, *s,* after the escape of a *Colpoda* which had been partially swallowed. *n,* nucleus; *c.v.,* contractile vacuole.

complete in from one to three minutes if the victim is not over twice as large as the *Didinium.*

There are at least four factors involved in this process: (1) The pull exerted by the seizing organ; (2) the suction produced by the active expansion of the body; (3) the extension of the oral opening out over the object; (4) the pressure produced by the contraction of the mouth when the object is nearly swallowed.

1. That the seizing organ travels to the posterior end of *Didinium* and remains attached for some time after the prey is swallowed was shown by Thon (1905, p. 298) by means of sections. In entire specimens mounted in glycerine it can be seen attached to organisms which extend more than half way down the interior of the body (see Fig. 8). The seizing organ returns to its former position after the food is partially digested. If *Didinia* are fed on *Colpoda,* it frequently happens that the ectosarc breaks immediately after the seizing organ is attached to it; then only a very small portion remains attached to the organ, which at once travels to the posterior end of the body drawing the bit of ectosarc with it. This portion of substance is frequently so small that it does not fill the space left by the indrawn organ and in such specimens one can clearly see a marked canal extending from the mouth to the posterior end of the animal, as represented in Fig. 18. The presence of this canal in some of the specimens studied by Balbiani led him to conclude that there is a permanent gut in *Didinium.* It is, however, nothing more than an invagination formed by the movement of the seizing organ and it disappears as soon as this organ returns to its accustomed position. In this canal the food lies. It is therefore in one sense merely in contact with the surface of the body after it is swallowed, much as in *Amœba* where the ectosarc surrounds the food particles and is taken in with it.

2. In some specimens the canal referred to above remains open to the exterior and fills with water as fast as it is formed. In others, however, the oral opening closes as soon as the organ is drawn in and this, of course, prevents the canal from filling. In such specimens, marked depressions are formed in the sides, especially near the anterior end, so that an end view of a *Didinium* in this condition sometimes presents an outline which

is nearly rectangular. This wrinkling of the outer surface indicates that there has been an active expansion; the outer layer of the body has become larger and no longer fits closely over the inner portion. It caves in owing to the decrease in internal pressure. During the swallowing process the extension of the outer layer produces a suction which tends to draw substance in. The fact that one frequently finds spaces filled with liquid (water vacuoles) between the object swallowed and the surrounding wall of the *Didinium,* also indicates that the internal pressure has been decreased. These water vacuoles can be accounted for by assuming that the decreased internal pressure causes the water to leak in around the object as it is passing the oral edge, or by assuming that it has been forced out of the substance already swallowed, owing to its subjection to a decrease in pressure. It is therefore evident that there is an active expansion in the outer portion of *Didinium* and that this makes the internal pressure less than the external and produces suction, which tends to draw the food into the body.

3. That the oral edge moves out to surround the organism captured is evident, but I was not able to ascertain whether this edge creeps out over the body during the swallowing process or whether it is pushed out by the active spreading and extension of the wall back of it.

4. Finally, direct observation shows that the contraction of the oral opening after the prey is nearly swallowed helps to draw over it the *Didinium,* which in this stage of swallowing has the form of a sac. The contraction of the oral opening also forces the prey in at the same time, thus stretching and expanding the thin walls of the *Didinium* more than would be possible if only the inherent spreading forces within these walls were active. The irregularity in the form of a *Didinium* after swallowing a large object supports this conclusion (Fig. 17).

The most marvelous phenomenon in this whole process is the movement of the seizing organ and the active spreading out of the substance of the *Didinium* in the form of a sac-like structure. I know of no other organism with the possible exception of *Hydra* that has the power of such extreme extension.

One can not work long with this unicellular form without being deeply impressed with the complexity of its structure, as well as with its unique habits. The differentiation of the seizing organ with its highly specialized function is remarkable in an organism consisting of a single cell. It shows what we are coming to realize more and more fully as the Protozoa are subjected to more detailed study; namely, that many cells are exceedingly complicated.

Amount and Kind of Food.

The number of *Paramecia* devoured by a *Didinium* in a day depends, of course, upon the size of the *Paramecia*. In all the experiments on this subject the *Paramecia* were between one and two times as large as the *Didinia*. Usually *Didinia* which had been without food for at least a day were selected and put with *Paramecia*. Then after they had swallowed one, they were isolated and later returned from time to time. In this way it was found that they take a *Paramecium* about every three hours and divide two or three times in twenty-four hours. None of the experiments on feeding were, however, continued for more than twelve hours. It is therefore impossible to say that the *Didinia* would feed at the rate mentioned for longer periods.

Didinia which have swallowed a *Paramecium* are, as already stated, frequently very irregular in form, owing to unsymmetrical extension of the body-wall (Fig. 17). As digestion proceeds, such specimens gradually become symmetrical again. I have frequently seen these ciliates capture prey when they were so gorged that they were still very much distorted. This shows that the seizing organ returns to the anterior end of the body long before the creature engulfed is fully digested.

The amount of food that *Didinia* eat depends upon the temperature and various other conditions. Under most favorable conditions they consume relatively enormous quantities. If they should take a *Paramecium*, averaging one and one half times their own size, every three hours, as the experiments described above indicate, in twenty-four hours they would consume a mass of substance having a volume twelve times as great as their own. I know of no other creatures that even approach these in their feeding capacity.

At different times during the progress of this work, *Didinia* were tested as to their ability to capture various organisms. These organisms may be divided into two groups: (1) those which were captured or attacked and (2) those which were not.

Group 1.—Paramecium caudatum, P. aureleus, Colpoda, various sp., *Colpidium,* various sp., *Vorticella,* sp. (?), *Frontonia,* sp. (?), *Nassula,* sp. (?).

Group 2.—Stentor cæruleus, Euglena, various sp., *Spirostomum,* various sp., rotifers, various sp., *Loxophyllum* sp. (?), *Paramecium bursaria, Oxytricha,* various sp., *Stylonychia,* various sp., numerous small flagellates, some identified and some not, *Paramecia* which had been killed by rapid heating.

Of those in the first group all but *Nassula* were at different times seen captured and swallowed by *Didinia. Nassula* was vigorously attacked, but every time the seizing organ became attached the connection was immediately broken by the discharge of trichocysts. I did not, however, work very much with these organisms. It may be that *Didinia* can master them under conditions different from those to which they were subjected during my observations. It will thus be seen that *Didinia* feed on various forms. *Paramecia,* however, seem to be essential to their well-being. I have never been able to get rapid multiplication in their absence.

Choice of Food.

If organisms from both groups mentioned above are present in a culture at the same time, only those belonging to the first group will be taken. Under such conditions it appears as though the *Didinium* had the power of selecting its food, and because of this apparent power of selection *Didinium* is frequently referred to in discussions on the choice of food in protozoa.

In the account of the process of feeding as given by Balbiani (see above, p. 97) it must be assumed that the *Didinia* can in some way distinguish different protozoa at a distance, for he says that these hunters discharge their darts even before they come in contact with the victim. Thon's description (1905, p. 201) also implies the ability to distinguish different infusoria, although not at a distance. "Sehr wichtig sind die Beobachtungen des Zustandes, wenn die Didinien aus Mangel an Paramaecien, gezwungen sind die Flagelleten zu jagen." From this quotation it must be concluded that the flagellates are taken only in the absence of *Paramecium*, never if both forms are present in the same culture. *Didinia* must, therefore, have some method of distinguishing between them.

The assumption of choice based upon Balbiani's description of course falls to the ground, since it has been shown (p. 98) that his observations were erroneous, and Thon unfortunately did not ascertain the species to which the flagellates mentioned belong. It is therefore impossible to repeat his experiments. In all the forms on which I worked it was, however, found that there is no apparent selection among the different forms in the first group. All of them, excepting *Nassula,* were at different times seen captured in the presence of *Paramecia*. In order to emphasize this point, let me quote from my notes written immediately after the observations were made. "On March 7 *Didinia* were put into a drop of solution on a slide containing numerous *Colpoda* and some *Paramecia*. Scarcely any of these creatures were attacked for an hour after the *Didinia* were added, although there were frequent collisions. Finally one *Colpoda* was captured and immediately after this, in the same vicinity, two more and also a *Paramecium*. It is evident then that *Colpoda* and various other forms are taken in the presence of *Paramecia*. This fact, however, does not exclude the possibility of choice even among these. It may be that while various organisms are captured in the presence of *Paramecia,* the number of these taken is relatively small. Whether this is actually true or not can be ascertained only by statistically comparing the number of successful contacts in the various organisms. It is clear that *Didinia* come in contact with their prey by chance. The question is, does the seizing organ adhere to one relatively more often than to another? However this may be, it is certain that *Didinia* come in contact with all sorts of objects and devour all such to which the seizing organ will adhere. The question then remains, why does this organ adhere to the surface of certain forms and not to that of others?

The following quotation presents Jennings' view (1906, p. 185): "On coming in contact with a solid object it (*Didinium*) stops, pushes forward against the object the conical projection which bears the mouth, and revolves rapidly on its long axis. The mouth is armed with a number of strong ribs ending in points, which apparently project a little from the cone bearing the mouth. When pushed forward against a soft organism, these points apparently pierce and hold it. The revolution on the long axis has the appearance of a process of boring into the body. The mouth now opens widely and swallows the prey. . . . The point which interests us at present is that *Didinium* reacts in the way described not merely to objects which may serve as food, but also to all sorts of solid bodies. In other words, the process is one of the trial of all sorts of conditions. On coming in contact with a solid, *Didinium* 'tries' to pierce and swallow it. If this succeeds, well and good; if it does not, something else is 'tried.' In a culture containing many specimens of *Didinium*, the author has seen dozens of individuals reacting in this way to the bottom and the sides of the glass vessel, apparently making persevering efforts to pierce the glass. Others 'try' water plants, or masses of small algæ, about which many specimens gather at times. Of course they get no food in this way. On coming in contact with each other, the animals react in the same way, often becoming attached to each other, and sometimes forming chains of four or five. But they never succeed in swallowing one another. They often try rotifers in the same way but the outer integument of these organisms is so tough that *Didinium* does not succeed in piercing it, and the rotifer escapes. *Stentor* and *Spirostomum* are often fastened upon, but usually escape, owing to their large size, great activity and rather tough outer covering. The reason why *Paramecium* is usually employed as food rather than other organisms is clearly due to the fact that when the *Didinia* try these, they usually succeed in piercing and swallowing them while with most other objects they fail."

It will thus be seen that Jennings emphasizes three factors in discussing the process of fastening onto the prey: (1) the rotation of *Didinium* on its long axis; (2) the size and activity of the organism attacked; (3) the toughness of the outer covering of these organisms.

1. There is no doubt about the fact that these creatures are frequently seen rapidly rotating on the long axis with the anterior end against objects of all sorts, but I feel very certain that this reaction has nothing to do with the process of capturing food, for I have repeatedly seen specimens which had just swallowed a *Paramecium* performing this very act. As a matter of fact, after having swallowed its prey *Didinium* ordinarily swims down and when it comes in contact with the bottom it remains there rotating on its axis, so that shortly after feeding one can frequently see many of them with the anterior end against the bottom, spinning much like miniature tops. Specimens which had just been fed were also repeatedly seen swimming about in contact with each other, sometimes as many as five or six in series, like a string of beads, each one apparently trying to seize the one in front of it.

One never, or rarely at least, sees this reaction in specimens which have been without food. Such specimens immediately respond with the motor reaction when they come in contact with an object. They appear to be darting about in every direction in wildest confusion, never coming to rest until they are actually attached to their prey. This attachment, as previously stated (p. 104), takes place at the very instant the *Didinium* strikes the surface to which it becomes fastened and never after having been in contact for some time apparently boring into the surface. The boring reaction may then be considered to be due to inactivity. *Didinium* responds in this way when the threshold of stimulation is increased to such an extent that contact no longer induces the avoiding reaction.

2. The size and the activity of the organisms are important factors in the general process of feeding, but they have nothing to do with the question as to the cause of the attachment of the seizing organ, for it readily adheres to *Frontonia* which are much larger than *Paramecia* and it does not adhere to *Euglenæ* and specimens of *Spirostomum,* which are much smaller.

3. The character of the outer surface seems to be the controlling factor in determining the possibility of the attachment of the seizing organ and this, together with the trichocysts and the size and activity of the organism, determines whether or not it

can escape the attack of *Didinium*. I am inclined to believe that the chemical composition as well as the physical constitution has to do with this process, that it is not merely the toughness of the integument but also the chemical composition of this covering that prevents the seizing organ from sticking. One thing is certain: it does not stick to the surface of any of the forms mentioned under group 1 (p. 113), and surely the outer covering of some of these, *e. g., Oxytrica, Euglena,* etc., is no tougher than that of *Paramecium* or *Vorticella*.

Is the conclusion of Jennings that *Didinia* try all objects and feed on any they can master, valid? I think there can be no doubt but that it is. This conclusion, however, has nothing to do with the question of conscious choice. Neither does the fact that the apparent choice has been explained mechanically show that *Didinium* is devoid of consciousness. For all that is known to the contrary, *Didinium* may be aware of the difference between different organisms. It may have certain sensations when it comes in contact with one surface and others when it comes in contact with another. I am not assuming that this organism is conscious, nor am I assuming that it is not. The question as to consciousness in this creature is open and should be left open until we have much more evidence bearing on it than we now have.

SUMMARY.

1. When *Didinium* comes in contact with an object, it usually responds with the avoiding reaction. In this response it always turns toward the same side, in spite of the fact that it is practically radially symmetrical.

2. It feeds entirely upon living organisms, principally *Paramecium,* but it has been known to devour also *Colpoda, Colpidium, Frontonia* and *Vorticella*. It captures these organisms by accidentally coming in contact with them in swimming about at random. The prey is held by means of the seizing organ, which in some way adheres to the surface when the contact is made.

3. There are no trichocysts discharged by the *Didinia*, they come from the victims; neither is the seizing organ thrust out at

the prey, nor is the prey paralyzed by poison injected through this organ.

4. The trichocysts function as organs of defence. They are discharged in great numbers when the seizing organ becomes fastened to the ectosarc. This forces the *Didinium* back mechanically and frequently breaks the connection, thus setting the victim free.

5. Well fed *Didinia* continue to divide for some time without food, becoming smaller and smaller until they are not more than one tenth the size of the original one.

6. Encysted *Didinia* are frequently found. The chemical composition of the solution in which they live seems to cause them to encyst, rather than lack of food, as maintained by Thon.

7. The apparent choice of food is due to the fact that the seizing organ will adhere to the surface of some organisms and not to that of others. The *Didinia* come in contact with all sorts of objects in their random swimming and attempt to swallow all of those to which the seizing organ will adhere.

LITERATURE CITED.

Balbiani, E. G.

1873 Observations sur le Didinium nasutum. Arch. d. Zool. Exp., Vol. 2, pp. 363–394.

Calkins, Gary N.

1901 The protozoa. New York. 347 pp.

Jennings, H. S.

1906 The Behavior of the Lower Organisms. New York. 366 pp.

Thon, Karel.

1905 Ueber den feineren Bau von Didinium nasutum, O. F. M. Protistenkunde, Vols. 5–6, pp. 280–315.

SOME NEW TYPES OF CHROMOSOME DISTRIBUTION AND THEIR RELATION TO SEX.[1]

FERNANDUS PAYNE.

INTRODUCTON.

Since the discovery of the "accessory chromosome" in *Pyrrhocoris,* by Henking, this chromosome or its equivalent has been described in many other Hemiptera (Paulmier, Montgomery, Wilson and others) and also in many other tracheates—Orthoptera (McClung, Sutton and others), Coleoptera (Stevens), Myriapoda (Blackman and Medes) and Odonata (Lefevre and McGill). Wallace and Berry have also described it in the Arachnida. Essentially, the "accessory chromosome" is a spermatogonial chromosome, which is without a synaptic mate, and which divides in but one maturation division. The end result is that one half of the spermatozoa receive one more chromosome than the other half. The spermatozoa are therefore dimorphic in respect to the number of chromosomes.

A second type of dimorphism of the spermatozoa was discovered by Stevens ('05) in *Tenebrio* and by Wilson ('05) in *Lygæus, Cœnus* and several other genera of Hemiptera, and was afterward found by Stevens ('08) in the Diptera. In this type, both classes have the same number of chromosomes, but differ in that one class contains a large idiochromosome and the other a small one. These observers showed that the class containing the small idiochromosome produces males and the other class females. Wilson likewise established the fact that in forms having an accessory chromosome, the spermatozoa that contain this chromosome are female-producing, the others male-producing. In respect to sex-production, the accessory chromosome is, therefore, identical with the large idiochromosome. The homologue of the small idiochromosome is lacking.

---

[1] My material was taken in large part by Professor E. B. Wilson on his collecting trip through the South and West, and it is due to this material that I have been able to make this comparative study. I have also received much valuable material from Rev. A. H. Manee, Southern Pines, N. C., besides collections which I have made in New Jersey, Massachusetts and Indiana. This material Mr. E. P. Van Duzee, of Buffalo, has kindly identified for me. Throughout the entire work, I have been much indebted to Professor Wilson for helpful suggestions and criticisms. I wish to thank Dr. Alexander Petrunkevitch for the privilege of working in his private laboratory at Short Hills, New Jersey, for five weeks during the spring of 1908. The U. S. Bureau of Fisheries also extended to me the privilege of working in the laboratory at Woods Hole, Massachusetts, during the summer of 1908.

The relation of the accessory chromosome to sex-production has been confirmed by the work of Montgomery ('06) on the Hemiptera heteroptera, of Stevens ('05 and '06) in the Coleoptera, of Boring ('07) on the Hemiptera homoptera, of Lefevre and McGill ('08) on *Anasa* and *Anax* (one of the Odonata), and of Wassilieff ('07) on the cockroach. It has also received confirmation in the recent work of Morgan ('08) and von Baehr ('08) in the parthenogenetic reproduction of *Phylloxera* and *Aphis.*

Wilson ('09) has described a third type in *Syromastes.* Gross ('04) showed that the accessory chromosome in this species is bivalent, but described and figured 22 chromosomes in both male and female cells. Wilson confirmed this for the male, but inferred that the female cells should contain 24 chromosomes or two more than the male, and he has since confirmed the correctness of this inference by direct observation. Hence, contrary to the assumption of Gross, both classes of spermatozoa must be functional, the class containing the bivalent accessory chromosome producing females, the others, males.

A fourth type was described by myself ('08) in a preliminary note on *Gelastocoris* (*Galgulus*). In this type there are five chromosomes which divide as univalents in the first maturation division. In the second division they do not divide, but four of the five pass to one pole and the other one to the opposite pole. In this form the female has three more chromosomes than the male, and the spermatozoa which contain the three extra chromosomes must therefore be the female-producing class.

A study of the Reduviidæ has brought to light several additional types here to be described. In *Diplocodus,* belonging to this family, occurs the second type with one large and one small idiochromosome. As in *Lygæus* and other forms, these divide as univalents in the first division, but in the second, they come together in the middle of the metaphase plate to form a dyad. The two chromosomes of this dyad group separate, one passing to one pole and the other to the opposite pole.

Another and the first new type described in the present paper is found in *Fitchia,* where there are three chromosomes which divide as univalents in the first division, but in the second division arrange themselves in the form of a triad group, two members of which pass to one pole and one to the other.

In *Prionidus* and *Sinea,* we find a second new type in which there are four corresponding chromosomes. These four divide only in the first division. In the second, they are arranged in the form of a tetrad group, three members of which pass to one pole and one to the opposite one.

A third new type is present in *Acholla multispinosa.* Here there are six univalent chromosomes in the first division. My evidence is not absolutely conclusive in this case, but as far as it goes, it indicates that these six divide in the first division, but in the second, five of them pass, without dividing, to one pole and one to the other.

In all these cases the male and female chromosome groups differ correspondingly. In *Diplocodus* the male and female cells have the same number of chromosomes, but differ in that the female cells have a large idiochromosome and the male cells a small one. In *Fitchia,* the female has one more chromosome than the male; in *Prionidus* and *Sinea* two; in *Gelastocoris* three; and in *Acholla multispinosa* four. These facts prove that in each case one class of spermatozoa is female-producing and the other class male-producing.

The discovery of these types adds nothing new in principle to the theory of sex production as put forward by Wilson ('06), but they are perfectly consistent with it. *Acholla multispinosa,* in which the male seems to have the larger quantity, offers evidence against the interpretation that sex is determined by a quantitative relation of the chromatin.

In the preliminary note ('08) on *Gelastocoris,* I used the term "differential chromosomes" to refer to the chromosomes of the pentad group. Throughout the present paper, the same term

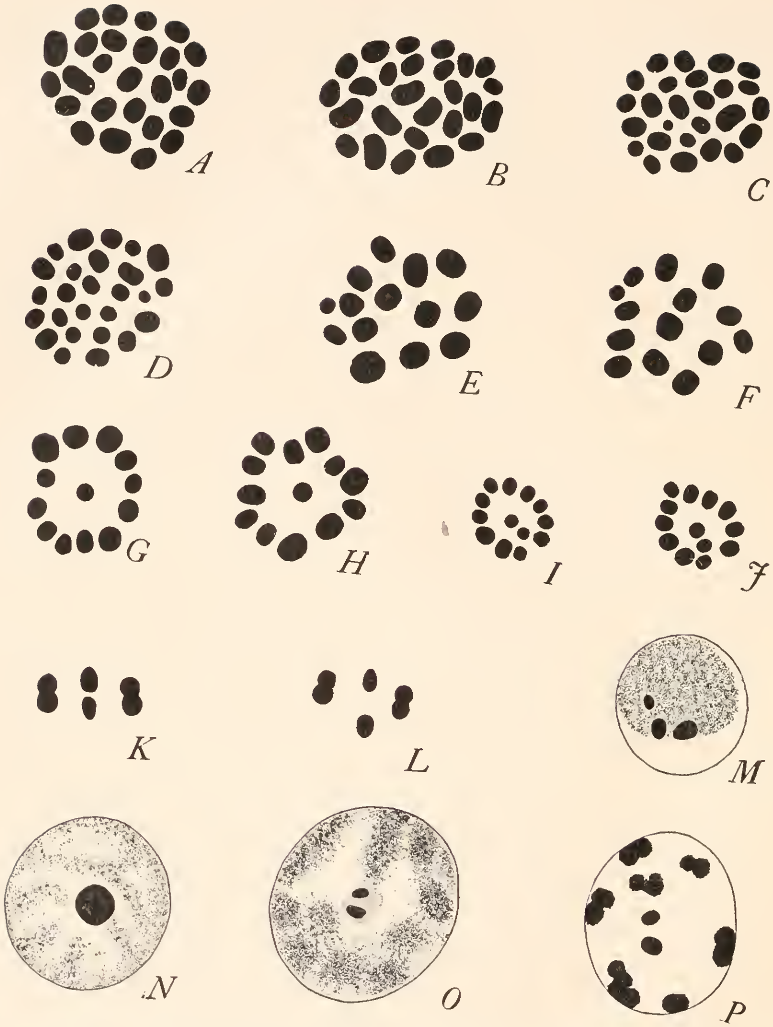


FIG. 1. *Diplocodus exsanguis* Stal. *A* and *B*, metaphase, polar view, of two female cells showing 26 chromosomes; *C* and *D*, metaphase, polar view, of two male cells, 26 chromosomes; *E* and *F*, two metaphase figures of the first maturation division, 14 chromosomes; *G* and *H*, two metaphase figures of the second maturation division, 13 chromosomes; *I* and *J*, two anaphases of the second division, taken from the same spindle; *K*, a side view of the second division, metaphase; *L*, side view of the second division, early anaphase, showing the early separation of the idiochromosomes; *M*, the early growth period, about contraction-phase. Two of the dark bodies are probably the idiochromosomes and the other the plasmosome; *N* and *O*, two drawings from approximately the same stage in the growth period. In *N* the plas-

will be used in reference to the chromosomes of the triad, tetrad, pentad and hexad groups.

Descriptive.

Diplocodus exsanguis Stal.

In *Diplocodus* we find a typical pair of idiochromosomes as described by Wilson ('05) for several species of Hemiptera. A description is included here mainly to show the range of variability in chromosome distribution found within the limits of a single family of Hemiptera. There are 26 chromosomes in the somatic groups of both male and female cells (Fig. 1, *A, B, C* and *D*). While there is very little difference in size in the idiochromosomes, there is one chromosome in the male group, noticeably smaller than the others, which without doubt, is the small idiochromosome. As the idiochromosomes are separate in the first division, the metaphase plate of the first division shows 14 chromosomes (Fig. 1, *E* and *F*), one more than half the spermatogonial number. In these figures, the small idiochromosome can be readily identified, but one cannot always be sure of the large one. Both divide in this division. In the second division, there is a rearrangement of the chromosomes, 12 taking up the position of a ring with the idiochromosomes, one above the other, in the center (Fig. 1, *I* and *J*, side views of the second division, metaphase). A polar view of the metaphase accordingly shows only 13 chromosomes. Wilson figures them as coming into close apposition at this time to form an asymmetrical dyad. While I have not examined a large number of cases in *Diplocodus,* they, here, seem not to fuse, but only to come very close together as shown in Fig. 1, *I*. Fig. 1, *J*, shows that their separation precedes the division of the chromosomes in the ring, as is also the case in the forms described by Wilson. Fig. 1, *K* and *L,* represent two anaphases taken from the same spindle.

The history of the idiochromosomes during the growth period has not been fully traced. In the contraction-phase (Fig. 1, *M*),

mosome stains intensely black, while in *O* it is pale and the idiochromosomes can be seen lying embedded in it; *P* is a prophase figure of the first division, showing the idiochromosomes in marked contrast to the looser masses of chromatin condensing to form the remaining chromosomes. The enlargement is 3,726 diameters.

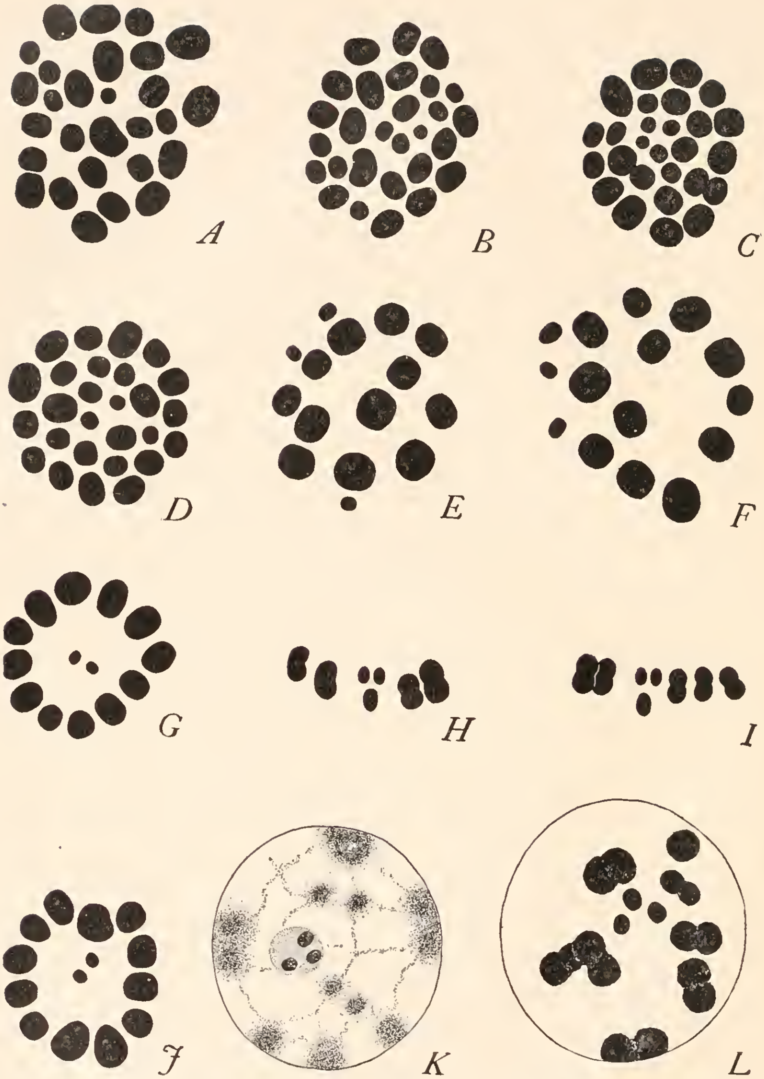


FIG. 2. *Fitchia spinosula* Stal. *A* and *B*, two female cells, metaphase, polar view, 28 chromosomes; *C* and *D*, two male cells, metaphase, 27 chromosomes; *E* and *F*, first maturation division, metaphase, showing 15 chromosomes —the three smallest ones are the differential chromosomes; *G* and *J*, polar views of the second division, metaphase—only two of the differential chromosomes are shown, as the third lies beneath these two; *H* and *I*, side views of the second division, metaphase—the triad group, formed by the three differential chromosomes, in the center; *K* gives the typical appearance of the plasmosome and differential chromosomes during the greater part of the growth

three dark bodies are sometimes present. Judging from the conditions found in Fig. 1, *N* and *O,* and from the relation of the chromosomes and plasmosome in *Prionidus,* where it has been fully worked out, it seems very probable that two of these dark bodies may be the idiochromosomes and the other, the plasmosome. Fig. 1, *N* and *O,* represent about the same stage in the growth period, but are entirely different in appearance. In *N,* the whole nucleolus stains intensely black, but again as shown in *O,* we see a pale plasmosome with the idiochromosomes embedded in it. There is no doubt but that the nucleolus in both cases represents the same thing. The only difference is in the staining capacity of the plasmosome. What causes this difference is impossible to say. However, it is very probable that the plasmosome is an active agent and as such is constantly undergoing changes, either chemical or physical, or both. In the prophase of the first division (Fig. 1, *P*) the plasmosome breaks down and the idiochromosomes stand out plainly while the other chromosomes are condensing. In the cases described by Wilson ('05) the idiochromosomes were attached to a plasmosome and not actually embedded in it as we find in *Diplocodus.*

Fitchia spinosula Stal.

In forms where the accessory chromosome is a single unpaired element, this chromosome remains throughout the growth period as a chromosome nucleolus, and since it divides in but one maturation mitosis, half the spermatozoa receive one more chromosome than the other. As already stated, these spermatozoa produce females, the others males, since the female somatic cells contain one more chromosome than the male.

In *Fitchia,* an exactly similar result is produced, yet, the manner in which the unequal distribution is brought about is entirely different. The female cells (Fig. 2, *A* and *B*) have 28 chromosomes, and the male cells (Fig. 2, *C* and *D*), 27. In neither of these groups can the differential chromosomes be recognized with certainty. The first division (Fig. 2, *E* and *F*) shows 15

period; *L*, prophase of the first maturation division—the plasmosome has disappeared and the chromosomes are forming. The enlargement is 3,726 diameters.

chromosomes, three of which are much smaller than the others, and prove to be the univalent differential chromosomes. Here they lie on the periphery. All the chromosomes divide equally as the same total number (15) is found in the metaphase plate of the second division. Now, however, the arrangement is different, the 12 larger chromosomes forming an irregular ring within which lie the three differential chromosomes in the form of a triad group, similar in arrangement to the pentad group in *Gelastocoris* (*Galgulus*). Two of these three, nearly or quite of the same size, lie in the same plane, while the third, which is a little larger, lies either above or below them on the other side of the equatorial plane (Fig. 2, *I* and *J*, two side views of the second division, metaphase). While my material does not show the anaphases of this division, the relations in the male and female cells and a comparison between this and the other two species, which show the same type of distribution and where anaphases are present, leave little doubt as to the manner of division. The 12 chromosomes in the ring divide equally, while the members of the triad group do not divide individually, but the group as a whole separates so that the two chromosomes pass to one pole and the one to the other. Two classes of spermatozoa containing respectively 13 and 14 chromosomes are thus produced. Since the female number is 28 and the male 27, it is evident that the reduced number in the egg must be 14, and that females are produced upon fertilization of the egg by the 14-chromosome class; males upon fertilization by the 13-chromosome class, as follows:

Egg 14 plus spermatozoön 13 = 27 (♂),
Egg 14 plus spermatozoön 14 = 28 (♀).

Although the end result is the same as in those species with an odd chromosome, this gives us a new type of chromosome distribution, which is the first step in a series, which bridges the gap between the relations found in *Diplocodus,* on the one hand, and *Gelastocoris* (*Galgulus*) on the other.

The history of the differential chromosomes in the growth period has not been followed in detail, but during the greater part of this time, a pale plasmosome is present and embedded in it are the three differential chromosomes as densely staining compact bodies (Fig. 2, *K*). In the prophase of the first division, the plasmosome disappears and the differential chromosomes remain (Fig. 2, *L*).

Rocconota annulicornis Stal.

With the exception of the size relations of the differential chromosomes, we find practically the same conditions in this form as in *Fitchia*. The spermatogonial cells (Fig. 3, *A* and *B*) show 27 chromosomes. Unfortunately no good female groups were present in my material, but since the relations are so similar to those found in *Fitchia*, there seems little doubt but that it contains one more chromosome than the male. In the first division, metaphase plate (Fig. 3, *C* and *D*), are 15 chromosomes, three of which are distinctly smaller than the remaining twelve, and which prove to be the differential chromosomes. All divide equally in this division. The characteristic regrouping in the second division again occurs. The 12 larger chromosomes form an irregular ring with the triad group, made up of the three differential chromosomes in the center (Fig. 3, *G* and *H*, side views of the second division, metaphase). Here all three differential chromosomes are practically of the same size. The chromosomes in the ring divide equally, while those of the triad group separate as in *Fitchia*, so that two pass to one pole and one to the other. The two classes of spermatozoa thus produced, are essentially similar to those of *Fitchia*, and are respectively male and female producing. Fig. 3, *I* and *J*, are two anaphases taken from the same spindle which show the unequal distribution of the chromosomes, 14 passing to one pole and 13 to the other.

Throughout the growth period, from shortly after synapsis to the prophase of the first division, there is present a distinct pale plasmosome, in which are embedded the three differential chromosomes (Fig. 3, *K* and *L*). The exact stage in which the plasmosome and chromosomes make their appearance could not be determined. Fig. 3, *M*, represents the prophase of the first division, where the plasmosome has disappeared. The differential chromosomes stand out in marked contrast to the looser masses of chromotin condensing to form the remaining chromosomes.

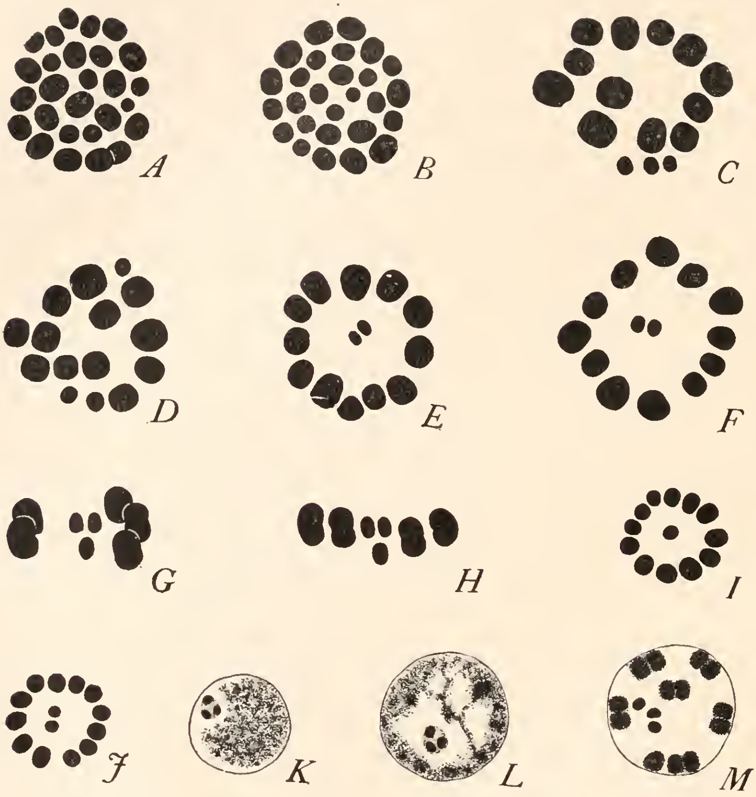


Fig. 3. *Rocconota annulicornis* Stal. *A* and *B*, male chromosome-groups, 27 chromosomes; *C* and *D*, polar views of the first spermatocyte division, showing 15 chromosomes—the three small ones are the differential chromosomes; *E* and *F*, polar views of the second division—only two of the three differential chromosomes are shown, as the third lies below the two; *G* and *H*, side views of the second division metaphase, showing arrangement of the triad group of differential chromosomes; *I* and *J*, two anaphases of the second division taken from the same spindle, which show the manner of separation of the triad group; *K*, a stage in the early growth period, showing the pale plasmosome and the three differential chromosomes embedded in it; *L*, a stage giving the typical appearance of the nucleus during the greater part of the growth period; *M*, prophase of the first division—the plasmosome has disappeared and the three differential chromosomes are easily recognizable. *K*, *L* and *M* are magnified 2,411 diameters; the remainder 3,726 diameters.

Conorhinus sanguisugus Lec.[1]

Conorhinus gives an additional verification of the type described in *Fitchia.* The number and size relations of the chromosomes are somewhat different. The spermatogonial divisions (Fig. 4, *A* and *B*) show 23 chromosomes, two of which are distinctly smaller than the others. In the metaphase plate of the first division (Fig. 4, *C* and *D*) there are 13 chromosomes, ten of which must be bivalent and three univalent as there are 23 in the spermotogonia. In this division, contrary to what is found in all other cases, the bivalent chromosomes form an irregular ring and the differential chromosomes, which are easily recognized by their size relations, take up a central position, but all lie in one plane. All the chromosomes divide equally in this division, so that each secondary spermatocyte receives 13 chromosomes, the arrangement of which is somewhat different from that in the first division. There are ten in the ring, but the differential chromosomes which occupy a central position, show a regrouping as in *Fitchia.* The large and one small one lie in the same plane, but the second small one is either above or below the other two, forming the characteristic triad group (Fig. 4, *G* and *H,* side views of the second division, metaphase). The ten chromosomes in the ring divide equally, while the chromosomes of the triad group separate, two going to one pole and the other to the opposite pole. Fig. 4, *I* and *J*, representing two anaphases from the same spindle, show the chromatin content of the two class of spermatozoa, which contain respectively 13 and 14 chromosomes.

The size relations of the differential chromosomes in the three forms illustrating this type of distribution are different in each case. In *Fitchia* the one which goes to the male-producing pole is slightly larger than the other two. In *Rocconota* all three are practically of the same size and in *Conorhinus,* one of the two which goes to the female-producing pole is larger than the other two.

The history of the differential chromosomes in the growth period has been followed as far as material permits. Sometimes

---

[1] This identification is somewhat doubtful as all my specimens are immature.

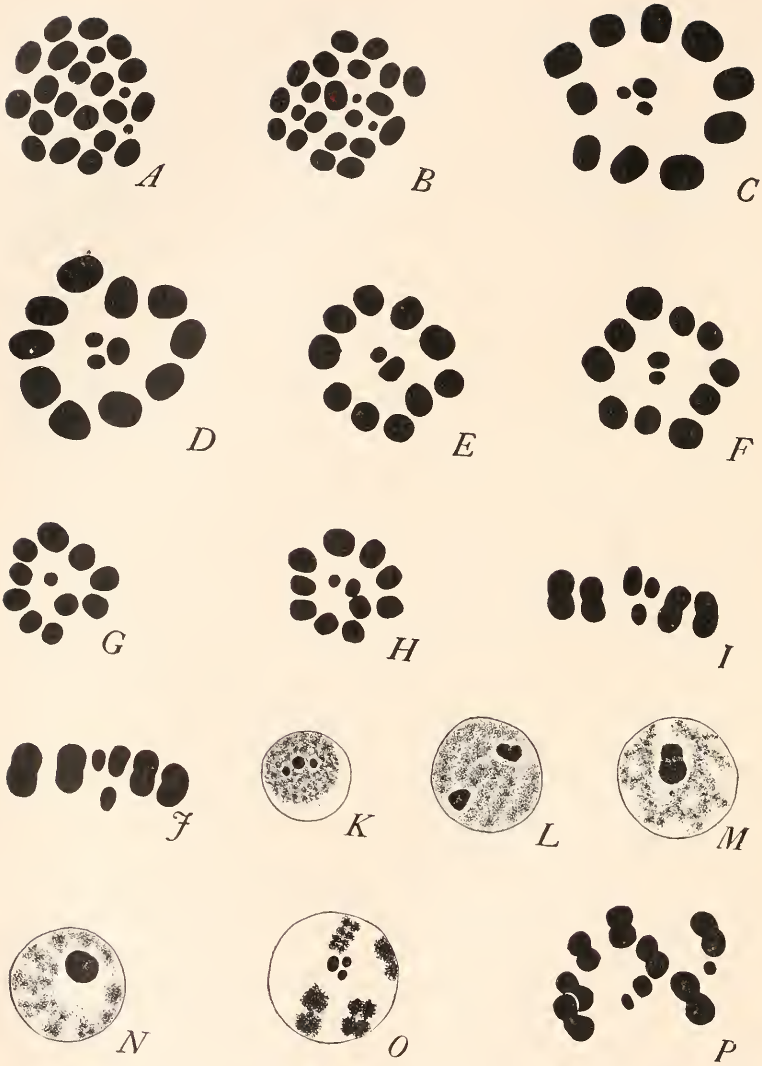


Fig. 4. *Conorhinus sanguisugus* Lec. *A* and *B*, male chromosome-groups, 23 chromosomes; *C* and *D*, polar views of the first maturation division, metaphase—the three differential chromosomes lie in the middle of the irregular ring; *E* and *F*, polar views of the second mitosis—only two differential chromosomes are shown; *G* and *H*, two anaphases of the second division taken from the same spindle and which show the manner in which the triad group separates; *I* and *J*, side views of the second division, metaphase, showing arrangement of the triad group; *K*, a stage in the early growth period, about contraction-phase—the three dark bodies are the three differential chromo-

they appear as separate and distinct individuals in the contraction phase (Fig. 4, *K*). A little later (Fig. 4, *L*) when the chromatin is scattered about through the nucleus, two dark bodies, nearly equal in size, are present. It is very probable that they are both plasmosomes. In one of them, three denser bodies can be faintly, yet unmistakably seen. These are no doubt the three differential chromosomes. The two plasmosomes seem to fuse into one body as in Fig. 4, *M*, and later condense into a somewhat spherical mass (Fig. 4, *N*), which persists throughout the greater part of the growth period. The plasmosome goes to pieces in the prophase of the first division and the differential chromosomes appear at the same time. While in Fig. 4, *M* and *N*, the differential chromosomes cannot be seen, there is little doubt but that they are present in the plasmosome. From these figures alone, it would be impossible to make the above statement, but in view of the condition in *Prionidus*, which is described later, I believe the correct interpretation has been given.

Prionidus cristatus Linn.

In his suggestive papers of '01 and '06, Montgomery described in *Prionidus*, the rest stage of the spermatogonia, the spermatogonial divisions and the rest stage of the primary spermatocytes. For the main part, my observations support his, but as we shall see, he was led to some erroneous conclusions because he did not follow the chromosomes through the first and second divisions. He figures 26 chromosomes in the spermatogonial divisions, and in describing these ('06) he says, "Of the 26 chromosomes two are much larger and two much smaller than the others. All these are found on careful inspection to be arrangeable into a series of pairs, in which the two components

somes; *L*, a stage after synapsis, showing two plasmosomes, in one of which the three differential chromosomes can be faintly seen; *M*, a stage a little later than *L*, showing fusion of the two plasmosomes; *N* shows the complete fusion of the plasmosomes and the typical appearance of the nucleus throughout the growth period; *O*, prophase of the first division—the plasmosome has disappeared—the three densely staining bodies are the differential chromosomes; *P*, a later prophase, just before the chromosomes arrange themselves in the equatorial plate. *K, L, M, N* and *O* are magnified 2,411 diameters; the remainder 3,726 diameters.

of each pair are of approximately equal volume except the two marked *K, k*. There is probably no monosome because the number is an even one."

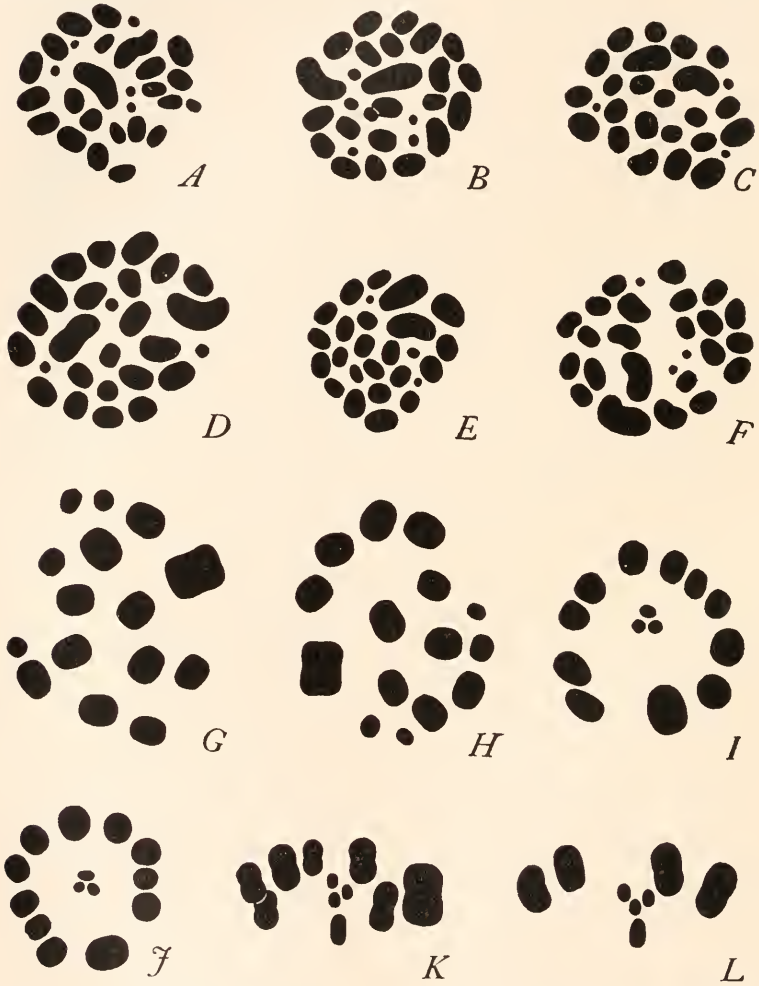


FIG. 5. *Prionidus cristatus* Linn. *A* and *B*, female cells showing 28 chromosomes—the six smallest ones are the differential chromosomes; *C, D, E* and *F*, male cells, 26 chromosomes—only three of the four differential chromosomes can be recognized with certainty—they are the three smallest ones; *G* and *H*, polar views of the first maturation division, 15 chromosomes—the four smallest ones are the differential chromosomes; *I* and *J*, polar views of the second division, metaphase—only three differential chromosomes are shown; *K* and *L*, side views of the second division, metaphase, showing the arrangement of the tetrad group. The enlargement is 3,726 diameters.

My figures of the male groups (Fig. 5, *C, D, E* and *F*) agree both in number and in size relations with those of Montgomery. There are 26 chromosomes, two of which are larger and three smaller than the rest. Although Montgomery's figures in both papers ('01 and '06) show three small chromosomes, he describes three small ones in the first paper and only two in the latter. The number of chromosomes and their behavior in the first and second divisions give an explanation of the size relations in the spermatogonia.

The female groups (Fig. 5, *A* and *B*) show 28 chromosomes, six of which are distinctly smaller than the others.

In the first maturation division metaphase, 15 chromosomes are present, four of which can be easily recognized as smaller than the remaining ones and one of the four is decidedly larger than the other three. There is no definite arrangement in these figures. All the chromosomes divide equally, so that accordingly each secondary spermatocyte receives 15 chromosomes, the arrangement of which is entirely different. The eleven larger chromosomes form an irregular ring while the four smaller ones, which prove to be the differential chromosomes, form a tetrad-group in the center (Fig. 5, *K* and *L,* two side views of the second division, metaphase). Unfortunately no anaphases of the second division were present, but the number and size relations in the male and female groups and the analogy between the behavior here and in *Sinea,* which is described later, leave no doubt as to the manner in which the tetrad-group separates. The eleven chromosomes in the ring divide equally, while the members of the tetrad-group do not divide, but the group as a whole separates so that the three smaller ones pass to one pole and the larger one passes to the other pole. Two classes of spermatozoa, containing 12 and 14 chromosomes, respectively, are thus produced. Accordingly the 14-chromosome class contains three small chromosomes while the 12-chromosome class would have none, as the differential chromosome which goes to this pole is as large as one half of one of the smaller dyads (see Fig. 5, *K* and *L*). If the 14-chromosome class meets the egg with 14 chromosomes, three of which are small, we would expect 28 chromosomes in the offspring, six of which should be small.

This number and the size relations are exactly what is found in the female cells (Fig. 5, *A* and *B*). Again, if the 12-chromosome class meets the same egg we would expect 26 chromosomes in the offspring, three of which should be small, and this number and relation is confirmed by the figures of the male cells (Fig. 5, *C, D, E* and *F*). From the facts at hand, it is evident that the reduced number of chromosomes in the egg is 14 and that three of these are decidedly smaller than the rest. Females must therefore be produced upon fertilization of the egg by the 14-chromosome class of spermatozoa; males by the 12-chromosome class.

Egg 14 plus spermatozoön 12 = 26 (♂),
Egg 14 plus spermatozoön 14 = 28 (♀).

From the above observations, it becomes apparent that the three small chromosomes and one other, which is not recognizable in the spermatogonial divisions, do not pair at synapsis. This gives an explanation of Montgomery's unequal pair.

Prionidus thus gives us a second new type of chromosome distribution and presents the intermediate stage between the types illustrated by *Fitchia* and *Gelastocoris*.

Montgomery has described only the rest stage of the growth period. In his earlier paper ('01) he says: "In the rest stage of the spermatocyte are found four chromatin nucleoli attached to the true nucleolus. One of these is longer than the others and rod-shaped." Later ('06) he describes this stage somewhat differently. "In the complete rest stage of the spermatocytes are found three or four safrininophilous bodies attached to the surface of a large more or less central plasmosome. They are of unequal volumes; and when there are three of them, each appears bipartite, while when there are four, the smallest are each unipartite. Perhaps as in *Sinea*, these relations are to be interpreted as three bivalent diplosomes, the smallest of which may sometimes have its parts separated."

I have followed the history of the differential chromosomes during the growth period somewhat in detail as the material proved favorable for this purpose. Between the last spermatogonial division and synapsis, a pale plasmosome persists. At the contraction-phase (Plate 1, *A*), there is present in addition to the plasmosome, a deeply staining body, which proves to be the first appearance of the differential chromosomes. In the spireme stage (Plate 1, *B*), shortly after synapsis, the two bodies are present, but the chromatic mass is more spherical. A little later (Plate 1, *C*), the chromatic body has become broken up and a plasmosome has formed around it. The next stage (Plate 1, *D*) shows the two bodies in contact and beginning to fuse. About this time the chromatic mass breaks up for the first time into its constituent elements, the four differential chromosomes, the larger of which is noticeably elongate (Plate 1, *E*). The two plasmosomes fuse completely to form a large more or less spherical one (Plate 1, *F*). Plate 1, *G*, shows the typical appearance of the nucleus throughout the greater part of the growth period. The larger differential chromosome shortens into a somewhat spherical form. All of them lie embedded in the pale plasmosome and not in contact with it as described by Montgomery. Nor do any of them, at any time, show an appearance of being bipartite and their later behavior in the maturation divisions shows conclusively that all four are univalent. As we shall see later, Montgomery's idea as to the relations in *Sinea* were incorrect, and this accounts in part for the wrong interpretation in *Prionidus*. In the prophase of the first division, when the chromatin is beginning to condense to form the chromosomes, the plasmosome disappears and the differential chromosomes remain.

In *Syromastes*, Wilson ('09) describes the same numerical difference in the male and female somatic cells as here described for *Prionidus*. The difference in that case, however, is brought about by two chromosomes which act as a unit and which divide in only one division.

Sinea diadema Fabr.

Montgomery ('01 and '06) described in part the behavior of the chromosomes in a species which he called "*Sinea diadema*," but the relations which I find in this species are entirely different from those which he described. There is, therefore, but little doubt that he studied a different species. This again em-

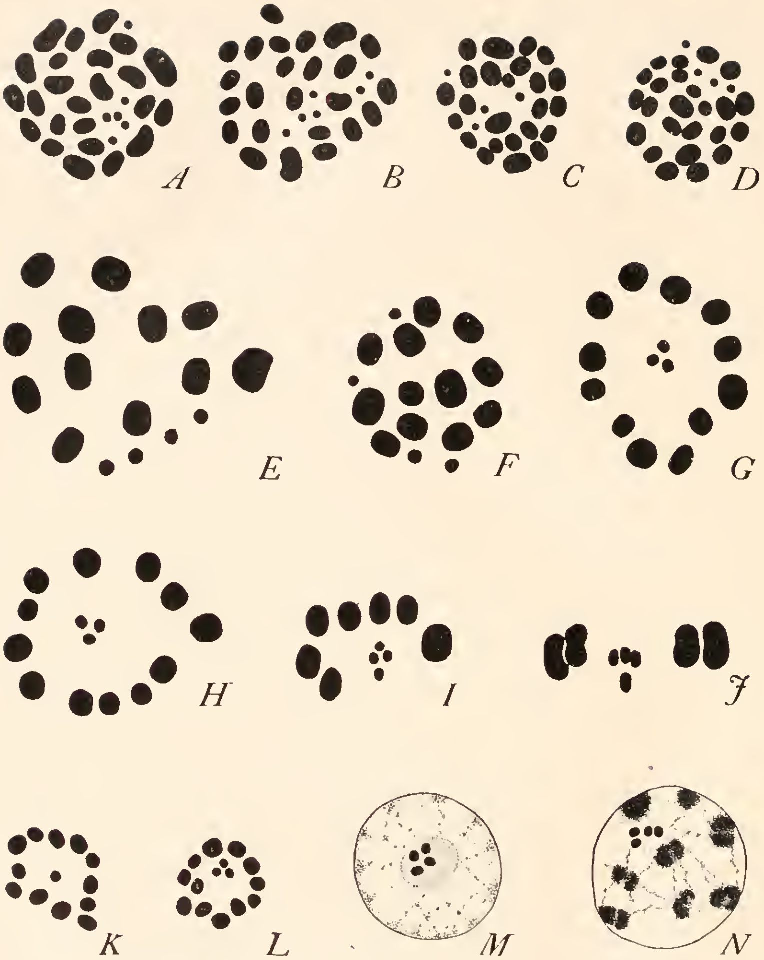


FIG. 6. *Sinea diadema* Fabr. *A* and *B*, female groups, metaphase figures, showing 30 chromosomes—the six small ones are the differential chromosomes; *C* and *D*, male groups, showing 28 chromosomes—the four small ones are the differential chromosomes; *E* and *F*, polar views of the first maturation division, metaphase, 16 chromosomes—the four small ones are the univalent differential chromosomes; *G* and *H*, polar views of the second division, metaphase—only three differential chromosomes are shown, as the fourth lies below the three; *I* and *J*, side views of the second division, metaphase, showing arrangement of the tetrad group; *K* and *L*, two anaphases of the second division, showing the manner in which the tetrad group separates, and the number of chromosomes in each of the two classes of spermatozoa; *M* gives the typical appearance of the plasmosome and differential chromosomes during the greater part of the growth period; *N*, a prophase of the first division after the plasmosome has disappeared—the four small condensed bodies are the differential chromosomes. *A–L* are magnified 3,726 diameters; *M* and *N* 2,411 diameters.

phasizes the necessity of keeping all specimens for future identification. It seems very probable that the species which Montgomery called "*Sinea diadema*" was *Acholla multispinosa,* for the first maturation division in *Acholla multispinosa* agrees exactly with that which Montgomery figured for *Sinea.*

The behavior of the chromosomes in *Sinea* is practically the same as in *Prionidus* and gives a beautiful confirmation of the results there described. In the female cell (Fig. 6, *A* and *B*), 30 chromosomes are distinctly visible, six of which are much smaller than the others. The male cells (Fig. 6, *C* and *D*) show 28 chromosomes, two less than the female number and four of these are smaller than the rest. There are 16 chromosomes in the metaphase of the first maturation division (Fig. 6, *E* and *F*), and since there are 28 chromosomes in the spermatogonia, 12 of these must be bivalent and four univalent. All the chromosomes divide equally in the first division. Hence the equatorial plate of the second division shows 16 chromosomes, but as usual, their arrangement is different. In the first division, the small chromosomes are peripheral, while in the second, they are central and are arranged in a tetrad-group, as are the four differential chromosomes in *Prionidus.* Fig. 6, *I* and *J,* show two side views of the second division, metaphase, with the tetrad group in the middle. In *Prionidus,* one chromosome of the tetrad group is larger than the other three, while in *Sinea* all four are practically the same size. In the second division, the chromosomes in the ring divide, while the tetrad group separates so that the three chromosomes above go to one pole and the one below to the other. Fig. 6, *K* and *L,* are two anaphases, showing the manner of separation. Two classes of spermatozoa are thus formed, containing 13 and 15 chromosomes, respectively. Judging from these facts, the reduced female group contains 15 chromosomes and females are produced upon fertilization by the 15-chromosome class of spermatozoa; males by the 13-chromosome class.

Egg 15 plus spermatozoön 13 = 28 (♂),
Egg 15 plus spermatozoön 15 = 30 (♀).

The size relations of the differential chromosomes which make

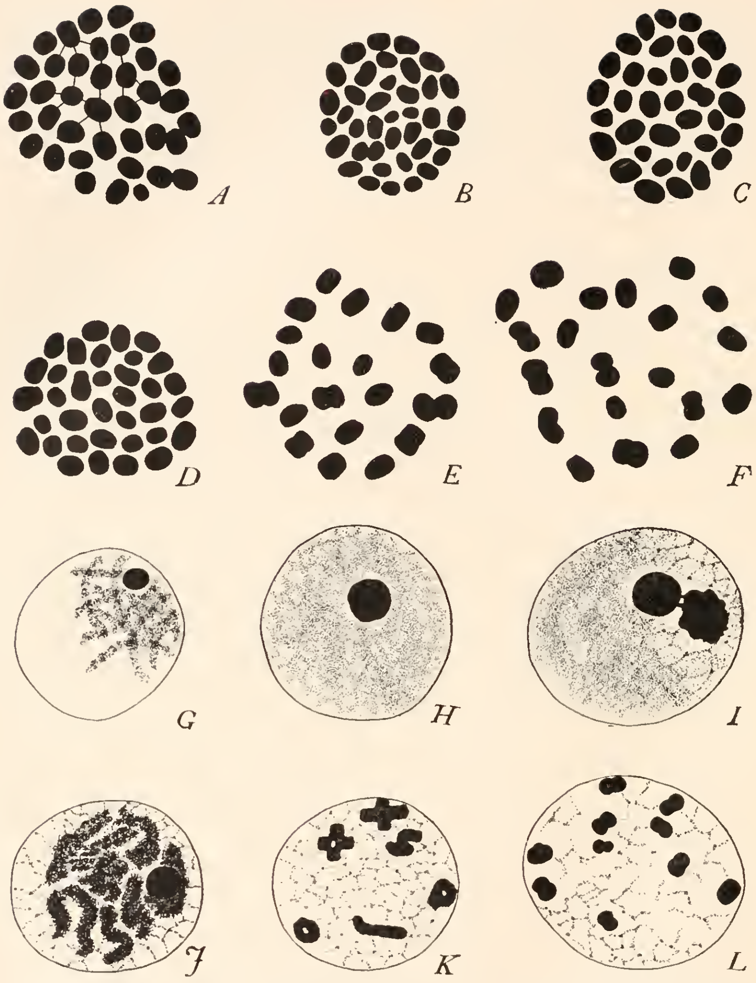


FIG. 7. *Gelastocoris* (*Galgulus*) *oculatus Fabr.* *A* and *B*, female groups, metaphase plates, showing 38 chromosomes; *C* and *D*, male groups, showing 35 chromosomes; *E* and *F*, polar views of the first maturation division, metaphase—the differential chromosomes are indistinguishable; *G*, a stage shortly after synapsis, showing the presence of the nucleolus; *H*, typical appearance of the nucleus and nucleolus throughout the growth period; *I*, a very early prophase of the first division—the contents of the nucleolus have been thrown out into the nucleus; *J*, prophase of the first division, when the chromosomes are just beginning to form—the plasmosome is still present, although apparently smaller; *K*, prophase of the first division—the chromosomes are condensing and the plasmosome has disappeared; *L*, late prophase of the first division—the chromosomes are formed, but have not taken up their position in the equatorial plate. *A*, *B*, *C* and *D* are magnified 3,105 diameters; *E* and *F* 2,009 diameters and *G*, *H*, *I*, *J*, *K* and *L* 1,297 diameters.

them easily recognizable in the male and female cells, help to confirm the above statement. Six are present in the female and only four in the male, and this is what we would expect, since one class of spermatozoa has three small chromosomes and other one.

The differential chromosomes have not been followed throughout the entire growth period. Fig. 6, *M*, shows the appearance of the nucleus during the greater part of this period. A large pale plasmosome is present in which are embedded the four differential chromosomes. The condition is almost exactly analogous to that found in *Prionidus.* In the prophase of the first division, the plasmosome disappears and the four differential chromosomes stand out clearly as condensed deeply staining bodies while the other chromosomes are condensing.

Gelastocoris (*Galgulus*) *oculatus* Fabr.

In a preliminary note ('08), I described a new type of sexual difference in the chromosome groups of *Gelastocoris oculatus.* At that time my material was limited and some of my conclusions were supported by only a few observations. Since then I have obtained new material and am now in a position to reaffirm all that was stated in the former paper. My figures of the first division showed among the chromosomes one or two minute dark bodies resembling yolk granules. As stated at that time, a doubt might be raised as to whether these bodies were chromosomes. However, my opinion as stated, was supported by the facts that the number of chromosomes in the prophase of the first division before the nuclear wall broke down, was always 20, and that the metaphase plate of the second division invariably showed 20 chromosomes. In my new material, I have found several metaphase plates which show 20 chromosomes without any granules whatever (Fig. 7, *K* and *L*). As the spermatogonial number is 35, 15 of the 20 must be bivalent and five univalent. There is no definite arrangement of the chromosomes in this division, but sometimes 15 are in a more or less irregular ring with the other five in the center, as is shown in Fig. 7, *L*. Whether these five are the same ones which form the pentad group in the second division is impossible to say. All the chromosomes divide equally so that each secondary spermatocyte receives 20 chromosomes. No reconstruction period follows. (Fig. 8 and 9 are the same as in the preliminary paper, but Fig. 7 is new with the exception of *A*.)

My former description of the second division seems adequate, and as I have nothing new to add, I will quote it as there described.

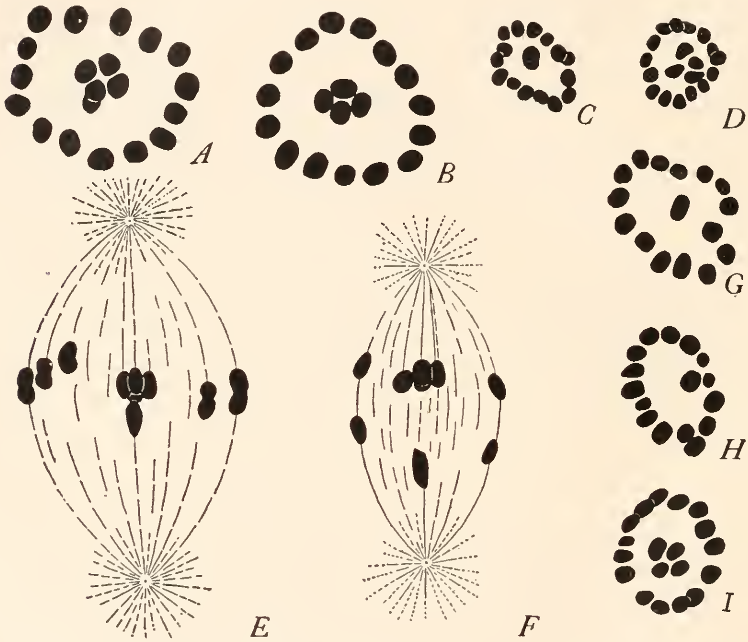


Fig. 8. *Galastocoris* (*Galgulus*) *oculatus* Fabr. *A* and *B*, metaphase figures of the second spermatocyte division, polar view, showing the ring of 15 chromosomes and the pentad group in the center—in *B*, the chromosome beneath the four group could not be shown without displacing it; *C* and *D*, late anaphases of the second division, polar view, showing the unequal distribution of the chromosomes; *E*, side view of the second division, metaphase, showing the typical arrangement and position of the pentad group; *F*, side view of the early anaphase, second division, showing the manner in which the chromosomes of the pentad group separate—the spindle in both *E* and *F* is diagrammatic; *G*, *H* and *I*, early anaphases of the second division. All drawings are made on the same scale and magnified 3,105 diameters.

"The second division which follows immediately after the first, shows a remarkable regrouping of certain of the chromosomes.

Fifteen of the 20 take up the position of a ring, within which is a definite compound element formed by the remaining five. These are now arranged in a pentad group, which always shows the same composition and occupies the same position. Four of these five chromosomes are grouped very closely together and lie in one plane, while the other one is either above or below this group of four, lying close to them on the other side of the equatorial plane (Fig. 8, *A*, polar view of the equatorial plate; Fig. 8, *E*, and Fig. 9, *C*, side views). The 15 chromosomes in the ring divide equally, while the chromosomes of the central pentad do not divide individually, but the group as a whole separates in such a manner that one chromosome passes to one pole and the other four to the other pole (Fig. 8, *F*, and Fig. 9, *D* and *F*). Two classes of spermatozoa are thus formed, which contain 16 and 19 chromosomes respectively. The early anaphase illustrating these two classes is shown in Fig. 8, *G*, *H* and *I*, and Fig. 9, *B*; the later anaphase in Fig. 8, *C* and *D*."

In my previous paper, I also stated somewhat cautiously that 35 was the number of chromosomes in the spermatogonia, as the count was made in only two cells. Fig. 7, *C* and *D*, shows two new groups and I have counted the chromosomes in several other cells, each case showing the same number. At present, I have, therefore, no hesitation is saying that 35 is the number of chromosomes found in the male cells. The female number (oögonial or follicle cells) is 38 (Fig. 7, *A* and *B*). From these facts it is evident that the reduced female group must contain 19 chromosomes; and that accordingly females are produced upon fertilization by the 19-chromosome class of spermatozoa; males upon fertilization by the 16-chromosome class.

Egg 19 plus spermatozoön 16 = 35 (♂),
Egg 19 plus spermatozoön 19 = 38 (♀).

During the growth period, between synapsis and the formation of the chromosomes preparatory to the first spermatocyte division, a large deeply staining body persists, which is more or less comparable to the nucleolus of the forms previously described. In the preliminary note, I suggested the possibility that this body contained the five differential chromosomes. Further study has thrown very little light upon the question. Fig. 7, *E. F. G* and *H,* represents its history as far as I have traced it. *E* shows it shortly after synapsis; *F* gives its typical apparance when the cell has reached its maximum size; *G* represents a stage toward the end of the growth period and before the chromosomes begin to form. At this time the contents are thrown out into the nucleus and it is probable that the part thrown out is chromatin as it appears to take part in the formation of the chromosome a little later. After the contents are thrown out, the nucleolus does not entirely disappear until the chromosomes are well formed (Fig. 7, *I*). As the five differential chromosomes cannot be identified before the second maturation division, it is impossible to trace their earlier history with accuracy. It is very evident, however, that if they are in the nucleolus they do not come out of it as condensed and separate individuals as they do in the other cases

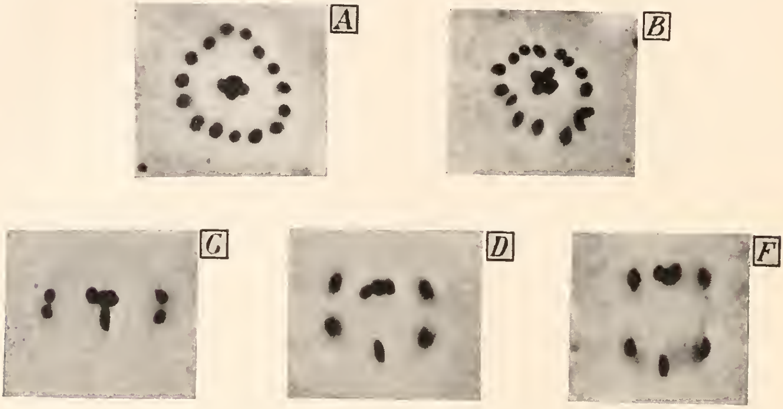


Fig. 9. *Gelastocoris* (*Galgulus*) *oculatus* Fabr. *A,* metaphase figure of the second division, polar view, showing the ring of 15 and the four chromosomes of the pentad group near the middle—the fifth chromosome of this group could not be shown, as it lies beneath the four; *B,* early anaphase of the second division, polar view, showing the 19 chromosomes which go to one pole; *C,* metaphase of the second division, side view, showing the typical position and arrangement of the chromosomes of the pentad group; *D* and *F,* anaphases of the second division, side view, showing the manner of separation of the pentad group, four chromosomes of which go to the one pole and one to the other—only three chromosomes of the four group show, as all of them do not lie in the same plane. The photographic enlargement is 1,500 diameters.

described. No direct evidence is at hand, yet I still think there is a possibility that the deeply staining body is a plasmosome in which are the five differential chromosomes. While reasoning by analogy may not always be a safe method, the above interpretation, it seems to me, is supported by a comparison of the nucleolus here with those in Fig. 1, *N* (*Diplocodus*), and Fig. 4, *N* (*Conorhinus*). In these two figures the nucleolus stains as intensely black as in *Gelastocoris,* yet it is evident that the differential chromosomes are present, although invisible.

Acholla multispinosa De Geer.

Montgomery has also described in part the spermatogenesis of a form which he called "*Acholla multispinosa,*" but according to Van Duzee's identification he was here again mistaken in the species. As previously stated, my figures of the first maturation division, metaphase, in *Acholla multispinosa,* agree in number and size relations with Montgomery's figures of the same stage in "*Sinea.*" Again his figures of "*Acholla multispinosa*" agree with my figures of the first division in *Acholla ampliata.* So it seems very probable that the species which he called "*Sinea diadema*" is *Acholla multispinosa,* and that his "*Acholla multispinosa*" is *Acholla ampliata.*

The behavior of the chromosomes in *Acholla multispinosa* is, in several respects, more remarkable than in any of the other species examined. The number of chromosomes in the female cells (Fig. 10, *A, B* and *C*) is 30, six of which are much smaller than the others. The male cells (Fig. 10, *D, E* and *F*) show 26 chromosomes, one of which is much larger and three much smaller than the remaining ones. At first sight, no evident relation is seen between the male and female groups. A relation becomes evident, however, from a study of the first and second maturation divisions. In the metaphase plate of the first division (Fig. 10, *H, I* and *J*) are 16 chromosomes. The three small ones are present and also the large one, which at this time is linked with two other chromosomes, one in contact with each end. This same grouping is present in the prophase of the first division (Fig. 10, *G*). Since there are 26 chromosomes in the

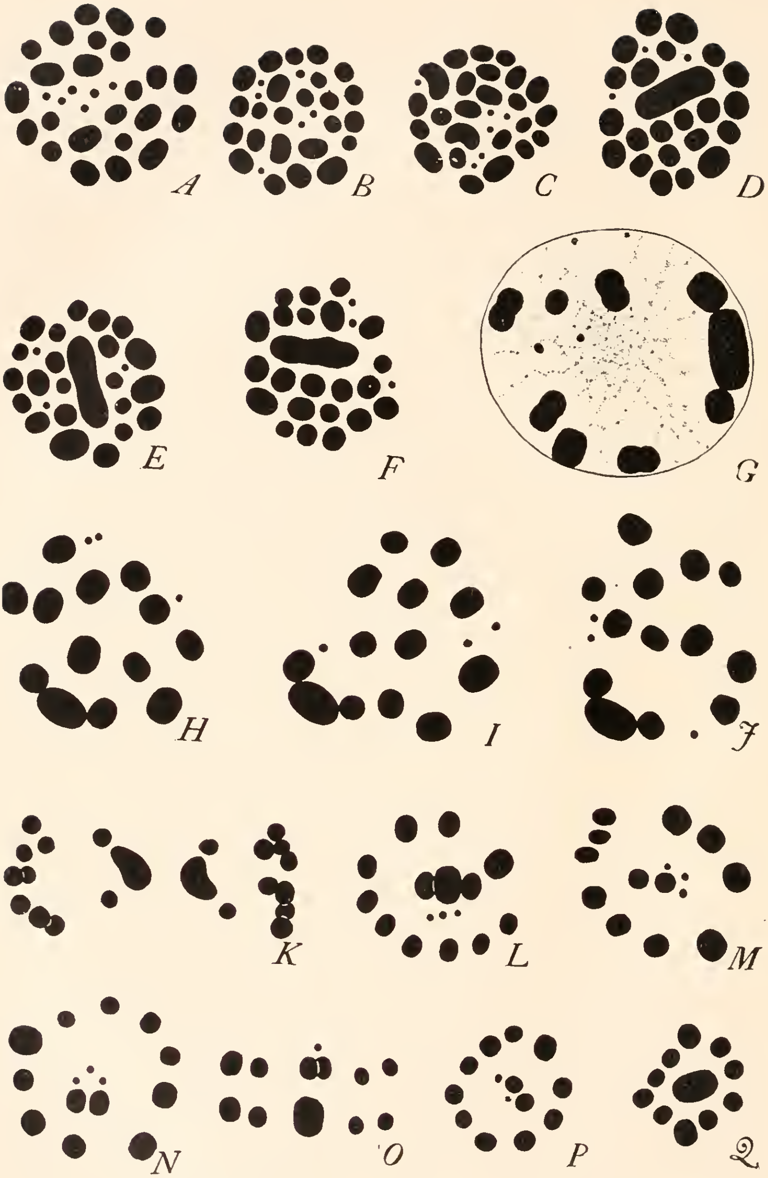


FIG. 10. *Acholla multispinosa* De Geer. *A–C,* female groups showing 30 chromosomes, six of which are small; *D–F,* male groups, 26 chromosomes, one of which is very large and three small; *G,* prophase of the first division showing the chromosome complex; *H–J,* metaphase figures of the first division with 16 chromosomes—the chromosome complex is still present; *K,* a side

spermatogonia and 16 in the first division, ten of these must be bivalent and six univalent. While I have not seen the anaphases showing the division of the three small chromosomes, it seems very probable that they and all the others divide equally in this division. The fact that 16 chromosomes are present in the equatorial plate of the second division, supports the above conclusion (Fig. 10, *L, M* and *N*; in *L* and *M*, the large chromosome which lies below the five in the middle, could not be shown). The chromosomes in the group of three formed by the large one and two others, lag behind and are the last to divide (Fig. 10, *K*, a side view of the first division, anaphase).

The metaphase plate of the second division again shows a regrouping of the chromosomes, similar in arrangement to those already described, but differing in the number of elements which compose the central group. Ten of the chromosomes form an irregular ring, within which are the other six, forming a hexad group (Fig. 10, *N*), composed of the three small chromosomes, the large one and the two which were linked with it in the first division. The three small and two medium sized ones lie approximately in one plane, while the large one lies a little above or below these five, on the other side of the equatorial plane. Unfortunately no side views of the metaphase showing all six members of the hexad group, were present in my material. The small chromosomes are so very minute at this stage that they are with difficulty seen at all. The chromosomes in the ring divide equally, while the members of the hexad group do not divide individually, but the group separates so that the large one passes to one pole and the remaining five to the other. Anaphases showing a clear demonstration of this separation were not present in my material. If there is any doubt as to the

view of the first division, anaphase, showing the late division of the complex; *L–N*, metaphase figures of the second division, showing the ring of ten bivalents and the univalents in the center—in *M* and *N* only five of the univalents are shown, as the large one lies beneath; *O*, side view of the second division, anaphase, showing the manner of separation of the large and two medium sized univalents—one small one is also present. No anaphases, showing the distribution of the three small univalents, were present. *P*, anaphase, polar view of the second division, showing the two medium univalents, and two of the small ones; *Q*, anaphase, showing the large univalent going to one pole. The enlargement is 4,018 diameters.

manner of the separation, it is in regard to the behavior of the small chromosomes. Fig. 10, *O*, a side view of an early anaphase, and Fig. 10, *P* and *Q*, pole views of the early anaphases, demonstrate clearly the behavior of the large and two medium sized ones. A number of anaphases showing one and two small chromosomes were present (Fig. 10, *O* and *P*), and when present, they were always with the two medium sized ones and not with the large one. A thorough study of many anaphases showing the large chromosome, has in no case, revealed the presence of a small one (Fig. 10, *P*). Further, the number of chromosomes and their size relations in the male and female cells makes it almost conclusive how the hexad group separates. As previously stated, the female group has 30 chromosomes, six of which are very small, and the male group has 26, three of which are small and one very large. If five members of the hexad group go to one pole and one to the other, two classes of spermatozoa are produced, containing 15 and 11 chromosomes respectively. The 15-chromosome class will contain 12 chromosomes of nearly equal size and three very small ones. The 11-chromosome class will contain ten chromosomes of approximately equal size and one very large one. Now, if the 15-chromosome class meets an egg with 15 chromosomes, three of which are small, the cells of the resulting individual will have 30 chromosomes, six of which will be small. This condition is fulfilled in the female chromosome group. If the same egg is met by the 11-chromosome class of spermatozoa, the cells of the offspring will contain 26 chromosomes, three of which will be small and one very large. This is the number and size relations found in the male cells. No other manner of separation of the hexad group could bring about a similar end result. It becomes evident then that the reduced number of chromosomes in the egg is 15, and that females are produced upon fertilization by the 15-chromosome class of spermatozoa; males upon fertilization by the 11-chromosome class.

Egg 15 plus spermatozoön 11 = 26 (♂),
Egg 15 plus spermatozoön 15 = 30 (♀).

As previously stated, the material which I have, does not justify me in saying that the above statements are absolutely true, but all the data point in that direction. I hope to reëxamine the form as soon as material can be procured. If, then, these inferences prove true, *Acholla multispinosa* gives another new type of chromosome distribution in which the female has four more chromosomes than the male. For lack of material, I have not attempted to follow the differential chromosomes through the growth period.

It was noticed in the prophase and metaphase of the first division that three chromosomes (the large one and two others) were joined together end to end. Montgomery described such a complex in his account of the chromosomes in a species which he called "*Sinea diadema*." In his first paper ('01) he considered the large chromosome representing two bivalents, but later ('06) described it as one large bivalent. All three members of this complex prove to be univalent in *Acholla,* since they divide only in the first mitosis.

In all previous cases where a dimorphism of the spermatozoa in regard to the number of chromosomes exists, the female has not only received the larger number of chromosomes, but as far as we can judge with the eye, the larger amount of chromatin. While the numerical rule still holds, *Acholla* seems to give an exception in that the quantity of chromatin which goes to the male-producing pole is greater than that which goes to the female-producing one. I have made a number of measurements in the first and second divisions, and in every case, the large chromosome seems to contain a larger amount of chromatin than the remaining five differential chromosomes. In connection with the question of sex-determination, this point will be discussed further.

What can be the significance of such a chromosome complex as is present in the first maturation division and in the prophase of this division? In the Phasmidæ, Sinety ('01) recorded a coupling of the odd chromosome with one of the ordinary bivalents. McClung ('05) extended this observation to two other families of Orthoptera, the Acrididæ and Locustidæ. Wilson ('07) describes a coupling of the supernumerary chromosome with one of the idiochromosomes in *Metapodius* and suggests the possibility that such chromosome couplings may give the physical basis of certain forms of correlation in heredity. The suggestion seems a very plausible one for the above cases, but the complex in *Acholla* is somewhat different. All three chromosomes are univalent and divide in the first division. In the second division the group separates, the large chromosome passing to one pole and the other two to the opposite pole.

Acholla ampliata Stal.

Acholla ampliata appears to be the species which Montgomery ('01 and '06) describes under the name of "*Acholla multispinosa*." He figures the spermatogonial division with 32 chromosomes, eight of which are very small, and both the first and second divisions with 16 chromosomes, four of which are small. In the first division, the four small ones may lie in any position, but in the second division, they are centrally placed and grouped closely together. Unfortunately my material shows only the first and second divisions which agree with those, which Montgomery figures, except that in the second division, the small ones are grouped so closely together that it is impossible to distinguish

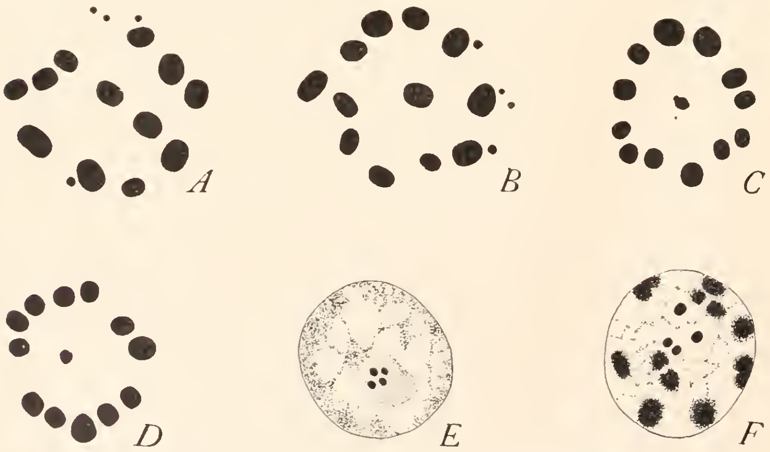
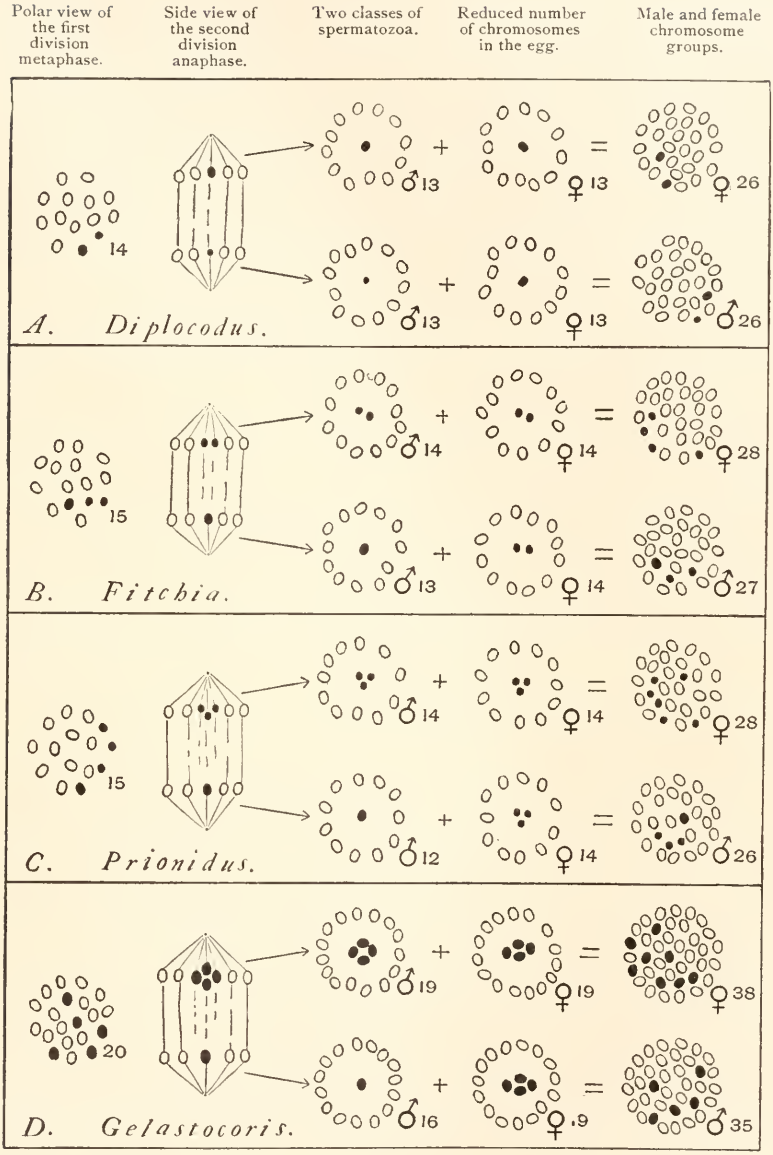


Fig. 11. *Acholla ampliata* Stal. *A* and *B*, metaphase figures of the first division, 16 chromosomes; *C* and *D*, metaphase figures of the second division—there are 12 chromosomes in the ring and the small ones are grouped in the center so that it is impossible to count them; *E*, typical appearance of the nucleus during the later part of the growth period—the four small chromosomes are embedded in the plasmosome; *F*, prophase of the first division—the plasmosome has disappeared, but the four small chromosomes remain. The enlargement is 4,018 diameters.


Polar view of the first division metaphase.
Side view of the second division anaphase.
Two classes of spermatozoa.
Reduced number of chromosomes in the egg.
Male and female chromosome groups.
14
♂13
♀13
♀26
♂13
♀13
♂26
A. Diplocodus.
15
♂14
♀14
♀28
♂13
♀14
♂27
B. Fitchia.
15
♂14
♀14
♀28
♂12
♀14
♂26
C. Prionidus.
20
♂19
♀19
♀38
♂16
♀19
♂35
D. Gelastocoris.

the number or arrangement with certainty. This difference, however, could be easily accounted for by a difference in fixation, as I have noticed the same thing in regard to the tetrad group in *Sinea.* As he found 32 chromosomes in the spermatogonia, Montgomery thought that the four small ones divided equally in both divisions, and he figured them as dyads attached to the plasmosome during the growth period. I have no evidence to offer to the contrary, but one thing comes out clearly in a study of the growth period, and that is that the four small chromatin bodies which Montgomery calls "chromatin nucleoli," show no appearance of being bivalent, and that they are not merely in contact with the plasmosome, but are actually embedded in it (Fig. 11, *E*). In the prophase of the first division (Fig. 11, *F*), the plasmosome breaks down and the small chromosomes remain, but even here they do not appear to be bivalent.

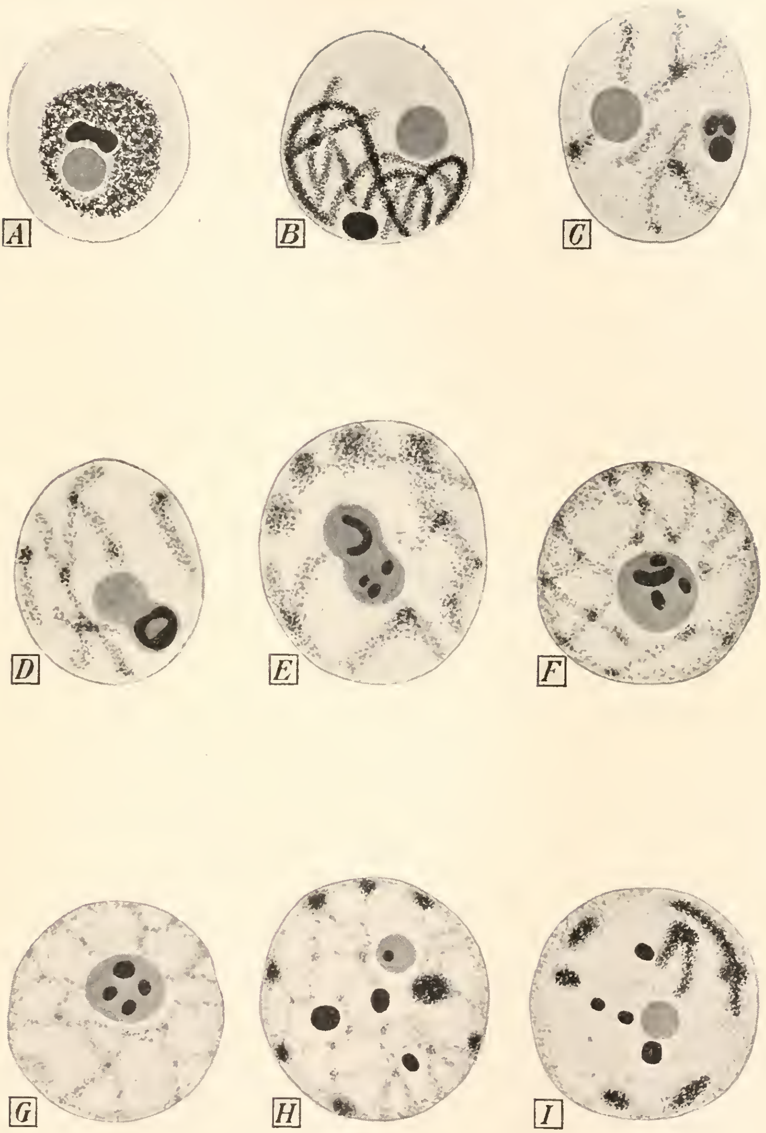
In comparing Fig. 11, *E* and *F*, with Fig. 6, *M* and *N*, the same stages in *Sinea diadema,* we notice a striking similarity. As the four small chromosomes embedded in the plasmosome in *Sinea* are univalent, is it not also possible that the same is true in *Acholla?* In the light of the present discoveries, it seems desirable that the form be reëxamined.

In order to summarize briefly the results, I have made a diagram (Fig. 12) illustrating the four different types of chromosome distribution described in the present paper. I have omitted from the diagram the type described in *Acholla multispinosa* as my evidence is hardly conclusive. The differential chromosomes are in each case made in black, while the remaining ones are left in outline. The size relations of the differential chromosomes are maintained as nearly as possible. In the first column to the left, are the metaphase figures, polar view, of the first division in each type. In the second column are side views of the second division, anaphase, showing the manner of separation of the dyad, triad, tetrad and pentad groups of differential chromosomes. The third column gives the two classes of spermatozoa produced in each type; the fourth, the reduced number of chromosomes in the egg (inferred); and the fifth, the end result produced by the fertilization of the egg by the two classes of spermatozoa.

(*To be continued.*)

Explanation of Plate I.

Prionidus cristatus Linn. These drawings illustrate the history of the differential chromosomes and the plasmosome during the growth period. *A,* about the contraction-phase of synapsis—the plasmosome and a deeply staining chromatic body is present; *B,* the spireme stage following synapsis; *C,* the chromatic body is breaking up and a plasmosome is forming around it; *D,* the stage following *C*—the two plasmosomes have commenced to fuse; *E,* the chromatic element breaks up for the first time into its constituent elements, the differential chromosomes; *F* shows the complete fusion of the two plasmosomes; *G* gives the typical appearance of the plasmosome and differential chromosomes during the greater part of the growth period—the larger differential chromosome has contracted; *H,* a prophase of the first division—the plasmosome has grown smaller and the differential chromosomes are coming out; *I,* prophase of the first division, where all the differential chromosomes have left the plasmosome. *A, B, C, D* and *E* are enlarged 4,018 diameters; *F, G, H* and *I,* 2,594 diameters.


A
B
C
D
E
F
G
H
I

Vol. XVI. *March, 1909.* *No. 4*

BIOLOGICAL BULLETIN

SOME NEW TYPES OF CHROMOSOME DISTRIBUTION AND THEIR RELATION TO SEX. *Continued.*

FERNANDUS PAYNE.

GENERAL.

The Chromosome Nucleoli.

The term "chromosome nucleolus" has come to mean, in a general sense, any chromosome, which remains condensed during the growth period. Under this heading, then, I shall discuss briefly the origin, valence and history of four different classes of chromosomes, namely: the accessory chromosome, idiochromosomes, m-chromosomes and differential chromosomes.

Henking ('91) observed a deeply staining body in the growth period of the primary spermatocytes of *Pyrrhocoris,* but did not detect its origin. He did recognize its chromatic nature and its unequal distribution in the second division, whereby one spermatozoön received one more chromatin element than the other. Montgomery ('98) was the first to detect that the accessory chromosome (chromosome nucleolus) was nothing more than a spermatogonial chromosome which differs in its behavior from the others, but he believed that it divided in both divisions. Paulmier, in his work on *Anasa tristis* ('99) described an accessory chromosome or what he called a "chromatin-nucleolus," which he thought originated at the time of synapsis by the union of two small spermatogonial chromosomes, divided in the first maturation division, but passed over undivided in the second. Montgomery ('01) discarded his former interpretation and accepted that of Paulmier. In the case of *Syromastes* ('04) and *Pyrrhocoris* ('06), Gross believed the accessory chromosome to arise by the fusion of two spermatogonial chromosomes which divided in one division but not in the other. According to these interpretations, then, the accessory chromosome would be bivalent.

McClung, however ('01), maintained the accessory to be univalent. Wilson ('05 and '06) has shown in the Hemiptera heteroptera that Paulmier ('99) and Montgomery ('01) were wrong in their interpretation of the origin of the accessory chromosome, that the small bivalent divides in both divisions, and that the accessory chromosome is a univalent spermatogonial chromosome which divides in only one division, and this was confirmed by Montgomery ('06) in opposition to his earlier conclusion.

The results of Gross ('04 and '06) have been more or less of a hindrance to the correct interpretation of the accessory chromosome. Wilson ('09) again has shown that Gross was mistaken in the case of *Pyrrhocoris* and that the accessory chromosome is here univalent. He confirms, however, Gross's results on the male cells of *Syromastes*. The spermatogonial number is even (22), and the accessory chromosome is here, in fact, bivalent, but it divides in only one division. Gross figured the same number of chromosomes in both male and female cells, but Wilson inferred (not having material) that the oögonial cells would show 24 chromosomes, since the accessory chromosome is bivalent. In an addendum to his last paper ('09*b*) Wilson states that his inference has proven true and that the female cells of *Syromastes* have, in fact, 24 chromosomes. In principle, therefore, *Syromastes* conforms to those forms in which the accessory chromosome is univalent.

Another contradiction occurs in the work of Foot and Strobell ('07) on *Anasa tristis,* who assert that the densely staining body in the rest stages of the spermatocytes is a true nucleolus and not a chromosome nucleolus formed by the accessory chromosome as described by Wilson ('05 and '06) and Montgomery ('06). Wilson's reëxamination of his preparations with additional studies on smear preparations and living material, led to the same conclusion as before. Lefevre and McGill ('08) have reëxamined the form and their results agree in every detail with those of Wilson and the later ones of Montgomery. From the evidence at hand, there seems to be no doubt that Foot and Strobell were in some manner misled.

Boring ('07), in 22 species of Hemiptera homoptera, describes the accessory chromosome as univalent and dividing only in the second division.

The work of McClung, Sinety and Sutton on the Orthoptera, has been consistent in showing that the accessory chromosome arises from a single spermatogonial chromosome. In apparent contradiction to this interpretation are the results of Voinov ('04) on *Gryllus,* Montgomery ('05) on *Syrbula* and Zweiger ('06) on *Forficula.* Each of these observers describe the accessory chromosome as arising from the fusion of two spermatogonial chromosomes and dividing in both divisions. In opposition to Montgomery's work on *Syrbula* is the recent paper by Robertson ('08) on the same genus. He seems to demonstrate conclusively that the accessory chromosome is univalent and divides in only one division. Randolph ('08) in the earwig *Anisolabis,* describes an equal pair of idiochromosomes. May it not be possible that Zweiger mistook, in *Forficula,* such a pair of idiochromosomes for a bivalent accessory?

Montgomery ('05) also described the accessory chromosome of *Lycosa,* a spider, as bivalent and arising from the union of two spermatogonial chromosomes. While he did not determine definitely the manner of its division, he was inclined to believe that it divided in both divisions, judging from the manner of its formation. About the same time Wallace ('05) published her account of *Agalena.* She figured two accessory chromosomes which divided in neither division. In an attempt to unravel these contradictions, Berry ('06) describes the behavior of the chromosomes in *Epeira.* She shows conclusively that there is an odd number of chromosomes in the spermatogonia and that the accessory chromosome is a spermatogonial chromosome which retains its identity throughout the growth period and divides in only one division. This makes the results in spiders consistent with the majority of those in the Orthoptera and Hemiptera.

The results of Blackman on *Scolopendra* ('05) and Medes on *Scutigera* ('05) show that the accessory chromosome in the Myriapoda is a single spermatogonial chromosome, but each finds the chromosome nucleolus of the growth period ("Karyosphere") made up of all the chromosomes.

McGill in her earlier work on *Anax junius* ('04) described the accessory chromosome as arising from a union of two small chromosomes as Paulmier had done in the case of *Anasa tristis.* The later paper of Lefevre and McGill ('08) on the same form, corrects the above error and shows that the accessory chromosome is a single spermatogonial chromosome.

The accessory chromosome in the Coleoptera as described by Stevens ('05, '06 and '09) arises from a spermatogonial chromosome and divides only in one division.

The idiochromosomes as described by Wilson ('05 and '06) for the Hemiptera, Stevens ('05 and '06) and Nowlin ('06) for the Coleoptera, and Stevens ('08) for the Diptera, are two univalent spermatogonial chromosomes which usually remain condensed throughout the growth period. They unite at synapsis in the Coleoptera and one chromosome nucleolus is present during the growth period except in *Tenebrio,* where the idiochromosome bivalent does not remain condensed. In the Hemiptera and Diptera they may or may not unite at the primary synapsis to form a bivalent body. In the former case, one chromosome nucleolus is present during the growth period, but separates into its univalent elements before the first maturation division. In the latter case, two chromosome nucleoli are present in the growth period.

The m-chromosomes as described by Wilson ('05), are two spermatogonial chromosomes which do not unite at the general synaptic period and which may or may not condense in the early growth period to form two small chromosome nucleoli. They undergo a late synapsis in the prophase of the first division to form a bivalent, which divides in both divisions.

We thus see that the three classes of chromosome nucleoli—namely, those derived from the accessory chromosome, from the idiochromosomes, and from the m-chromosomes, are alike in that all are direct descendents of spermatogonial chromosomes.

The differential chromosomes or chromosome nucleoli of the Reduviidæ, which are described in the present paper, are in each case, with the possible exception of *Acholla ampliata,* univalent, and derived from univalent spermatogonial chromosomes. The case of the large nucleolus of *Gelastocoris* is not so clear, but as previously stated, there is a possibility that the differential chromosomes are embedded in the nucleolus, although not as distinct and separate individuals as in the Reduviidæ. The differential chromosomes in the Reduviidæ, are without exception (as far as examined) embedded in a plasmosome. This plasmosome may be pale (*Prionidus, Sinea*) in which case the chromosomes stand out clearly, or it may stain rather densely (*Diplocodus, Conorhinus*) so that the chromosomes can be seen only faintly or not at all. In this respect they differ from the chromosome nucleoli formed by the accessory, m- and idiochromosomes. The accessory chromosome is, in the early part of the growth period, in contact with a pale plasmosome, but later separates from it. The idiochromosomes also may be associated during a part of the growth period with a plasmosome. The m-chromosomes may lie in any position and have no relation to the plasmosome.

Origin of the Differential Chromosomes.

In a study of the differential chromosomes, one of the most interesting questions which arises, is the origin of the asymmetrical chromosome distribution. It seems almost a certainty that these peculiar forms of distribution have not been present since the origin of the species, but have arisen sometime within its history.

In a discussion of this question, Montgomery ('06) is inclined to the belief that the m-chromosomes and idiochromosomes are not radically different structures, but are rather extremes of a series of modifications. Of their origin he says, "First a pair of autosomes (chromosomes) became modified so as to retain their compact nature during the growth period, still maintaining their approximate equivalence in volume. Because such allosomes are usually very small, we might conclude also, that they arose from the smallest pair of autosomes. In the next change would appear a growing disparity in size, which, if our last assumption be correct, would be due not to one becoming smaller and the other becoming larger, but rather to one retaining its original volume and to the other becoming much larger. This second step would then be one of differentiation of the two, a becoming different, probably implying also difference of metabolic activities. This would account for the lessening affinity of the two as exhibited by the protraction of the time of conjugation. Then would be attained the stage of the second type of diplosomes (idiochromosomes), no longer united but separate in the first maturation spindle. And the last step would be that, instead of a reduction division of them in this spindle there would take place there an equational division of each." This conception is rendered untenable by Wilson's discovery that in *Metapodius* a typical pair of m-chromosomes and of unequal idiochromosomes coexist in the same species. In his papers of '01 and '05, Montgomery has argued that the accessory chromosome is bivalent and arose by some abnormality in mitosis as by the failure of two spermatogonial chromosomes to separate. Wilson ('05 and '06) suggests that the accessory chromosome may have arisen by the gradual disappearance of the small idiochromosome. Montgomery ('06) realizes this possibility, although he offers objections to it. While Wilson still believes that this interpretation may be applicable in many cases, in his work on *Metapodius* ('09*b*), he gives another possibility of its origin along with that of the supernumeraries. He says, "This was suggested by the observation that in a very few cases in 22 chromosome individuals, both idiochromosomes were seen passing to the same pole in the second division. The rareness of this occurrence shows that it is doubtless to be regarded in one sense as abnormal. But even a single event in an original 22-chromosome male, if the resulting spermatozoa were functional, might give the starting point for the whole series of relations observed in the genus, including the establishment of an unpaired idiochromosome. The result of such a division should be a pair of spermatozoa containing respectively 10 and 12 chromosomes. The former might give rise at once to a race having an unpaired idiochromosome and the somatic number 21 in the male. The latter might similarly produce an individual having in the first generation a single supernumerary chromosome and in succeeding generations an additional number."

In regard to the possible origin of the supernumeraries in

Diabrotica, where they are associated with an odd chromosome instead of a pair of idiochromosomes, Stevens ('08) makes the following suggestion: "It may be possible, as was surmised earlier in the study, that (1) there will prove to be two distinct types (varieties or species), in each of the present species, one having the large unpaired heterochromosome only; the other having an equal pair of chromosomes as in *Haltica,* and that (2) the irregularities in time of division and the consequent peculiarities in number and distribution of the supernumeraries in *Diabrotica* are to be attributed to hybridism."

The differential chromosomes in *Gelastocoris* and in the Reduviidæ seem to have undergone a somewhat different history from that of the other types. It is clear that the single large idiochromosome of *Diplocodus* is represented in *Fitchia* by two chromosomes, in *Prionidus* and *Sinea* by three and in *Gelastocoris* by four chromosomes. It is very probable that the condition seen in *Diplocodus* is the most primitive one, and the essential question is how the other types can have been derived from this. Taking as an example *Fitchia,* where the female number is 28 and the male 27, if a pair of idiochromosomes was the original condition in the species, the original number of chromosomes must have been 26 in both male and female. If, in the male, the original large idiochromosome should break up into two parts, two classes of spermatozoa would be found, one containing 13 chromosomes and the other 14. All the eggs would have 13 chromosomes. If an egg were fertilized by a 14-chromosome class of spermatozoa, the result would be a female with 27 chromosomes. In maturation, this female would produce two kinds of eggs, one with 14 chromosomes and the other with 13. The 14-chromosome class fertilized by a spermatozoön with 13 chromosomes would produce either a male or female with 27 chromosomes. From these individuals, then, would arise both eggs and spermatozoa with 13 and 14 chromosomes. As the 14-chromosome class of spermatozoa would be female-producing, females with 28 chromosomes would be produced upon fertilization of an egg with 14 chromosomes by a spermatozoön with 14 chromosomes. Further, an egg with 14 chromosomes, fertilized by a spermatozoön with 13 chromosomes would produce a male with

27 chromosomes. This gives the number relations as we find them at present if the individuals with the original number (26) and the females with 27 disappear. The same explanation holds for the remainder of the types if we assume that the large idiochromosome breaks up into three or four elements instead of two. Another thing which adds weight to the above interpretation is the fact that the behavior of each of the triad, tetrad and pentad groups, as a whole, is exactly comparable to the behavior of a pair of idiochromosomes. Evidently the original irregularity might have first occurred in either sex and have been transferred to the other. This gives another method by which the chromosome number may change during the history of the species. While *Metapodius* and *Diabrotica* are species where a change is actually taking place at the present time, the forms described in the present paper (with the exception of *Diplocodus*) show a fixed condition after a change has taken place.

Sex Determination.

In a preliminary note ('08) Morgan has given some interesting data in regard to sex determination in phylloxerans, where all the fertilized eggs produce females and where both males and females develop from parthenogenetic eggs. In describing the spermatogenesis he says: "The reduced number of chromosomes is three. In the first spermatocyte division two of these divide equally, but the third lags behind the others, and finally in the very last stages of this division, it retreats to one of the poles. Thus there are three chromosomes in one of the two daughter cells, and only two in the other. Still more significant is the fact that the cell with the fewer chromosomes is very small; it contains very little cytoplasm and subsequently degenerates without forming a spermatozoön. In the second spermatocyte division all three chromosomes of the larger cell divide equally, thus producing two spermatids with three chromosomes each. These spermatids become spermatozoa. They correspond in their mode of development to the 'female-producing' spermatozoa of other insects. Hence we can understand why all fertilized eggs become females."

To those who have sought to bring forward a theory of sex determination of universal application, the parthenogenetic forms have been more or less of a stumbling block. The above results are in perfect harmony with the hypothesis of sex-production advocated by McClung, Stevens and Wilson.

Morgan also gives some further observations, indicative as to the manner in which males and sexual females are produced from parthenogenetic eggs. " I find that the somatic cells of the males of the species referred to above contain only five chromosomes. These five give in the spermatogenesis the reduced number three by two uniting with each other and the third having no partner. I find that the somatic cells of the female contain six chromosomes. It follows that at some time in the life cycle of the parthenogenetic eggs, one chromosome disappears in those eggs that become males, while the full number is retained in the female. It seems plausible that this change takes place in the formation of the single polar body given off by the parthenogenetic egg.

" The results seem to show that while the sex of the stem mother is connected with the presence of ' female-producing ' spermatozoa, the production of males and of sexual females is dependent on a process that takes place in the egg analogous to the same process that takes place in the spermatogenesis of other kinds of insects. Hence it follows that the egg as well as the sperm has the power of determining sex by regulating the number of its chromosomes."

Von Baehr's recent results ('08) and also those of Stevens ('09) on the aphids confirm those of Morgan on the phylloxerans.

In a preliminary note ('08) Baltzer gives some results on echinoderm eggs, where he finds in the early cleavage stages of fertilized eggs of *Echinus microtuberculatus* and *Strongylocentrotus lividus*, an unpaired element which comes from the egg nucleus. Further, this unpaired element is present in only a part of the eggs. He suggests the possibility that this may be a chromosome which has to do with sex determination. If this element should prove to be a chromosome and in one half of the eggs, it would be the reverse of the condition found in the insects, for there the unpaired chromosome has arisen in the male. However, two such diverse phenomena are not impossible in different groups of animals, as we have no reason to suppose that sex differentiation has taken place in the same manner in all animals. In fact, the quotation from Morgan shows that two classes of eggs are produced in phylloxerans and that the inequality of the chromosome distribution is accomplished in some manner at the time of the formation of the polar body.

McClung, Stevens and Wilson in their studies on the chromosomes of the insects have argued that the accessory chromosome and the idiochromosomes are either sex determinants or are in some manner connected with the determination of sex. The manner in which this is brought about yet remains a question. Wilson ('06) gives two interpretations by which the end result as we find it may be reached. First he follows out the earlier hypothesis of Castle ('03) that sex production may be interpreted as the result of a Mendelian segregation, transmission and dominance of the sexual characters. This interpretation involves two assumptions, neither of which have been proven, but which he argues may not be insuperable difficulties. One is the assumption that there are two kinds of eggs, the other, that selective fertilization occurs.

As an alternative point of view, he gives a second interpretation, in which he suggests that possibly the differential chromosomes may not be qualitatively different except in the degree of their special activity; or the end result may be due merely to a quantitative difference in the chromatin. Wilson, himself, in his work on *Metapodius* ('09), where the female may or may not have a larger amount of chromatin than the male, has brought forward evidence against the quantitative interpretation.

My results in the present paper offer nothing new in regard to the theory of sex determination, but are in perfect accord with the majority of previous results on the insects. I shall not venture far into theoretical discussions. There is, however, one question which naturally arises. Why do we have so few as one and two chromosomes involved in the production of males and females respectively in those forms with an odd chromosome, and as many as five and eight in *Gelastocoris?* This is not so strange, if the unequal distribution in these forms has arisen from a pair of idiochromosomes as previously explained. In every case whether the difference in number be one, two, three or four, the female has the larger quantity of chromatin with the possible exception of *Acholla multispinosa*. Here the male seems to have the greater quantity and this inequality is brought about by the differential chromosomes themselves. This argues further against the interpretation that sex is determined by a quantitative relation of the chromatin.

Zoölogical Laboratory,
Columbia University,
December 16, 1908.

LITERATURE.

Arnold, G.

'08 The Nucleolus and Microchromosomes in the Spermatogenesis of Hydrophilus piceus (Linn.). Archiv für Zellforschung, Band II., Heft 1.

Baehr, W. B. von.

'08 Über die Bildung der Sexualzellen bei Aphididæ. Zoologischen Anzeiger, Bd. 33, Oct., 1908.

Baumgartner, W. J.

'04 Some New Evidence for the Individuality of the Chromosomes. Biol. Bull., 8, 1.

Baltzer, F.

'08 Über die Grösze und Form der Chromosomen bei Seeigeleiern. Verhandlungen der Deutschen Zoologischen Gesellschaft. Vierte Sitzung.

Blackman, M. W.

'03 The Spermatogenesis of the Myriapoda. 2. On the Chromatin in the Spermatocytes of Scolopendra heros. Biol. Bull., Vol. 5.

'05 The Spermatogenesis of Scolopendra heros. Bull. Mus. Comp. Zoöl., Harvard, 48.

Berry, E. H.

'06 The "Accessory Chromosome" in Epeira. Biol. Bull., XI., p. 193.

Boring, A. M.

'07 A Study of the Spermatogenesis of 22 Species of the Membracidæ, Cercopidæ and Fulgoridæ. Journ. Exp. Zoöl., IV., 4.

Castle, W. E.

'03 The Heredity of Sex. Bull. Mus. Comp. Zoöl., Harvard, Vol. 40, No. 4.

Foot, K., and Strobell, E. C.

'07 The "Accessory Chromosome" of Anasa tristis. Biol. Bull., XII., p. 119.

'07a A Study of the Chromosomes in the Spermatogenesis of Anasa tristis. Amer. Journ. Anat., 7.

Gross, J.

'04 Die Spermatogenese von Syromastes marginatus. Zool. Jahrb., Anat. und Ontog., XX., 3.

'06 Die Spermatogenese von Pyrrhocoris apterus. Zool. Jahrb., XXIII., p. 269.

Henking, H.

'91 Ueber Spermatogense und deren Beziehung zur Eientwicklung bei Pyrrhocoris apterus. Zeitschr. Wiss. Zool., 51.

Lefevre, G., and McGill, C.

'08 The Chromosomes of Anasa tristis and Anax junius. Amer. Journ. Anat., Vol. VII., No. 4.

McClung, C. E.

'99 A Peculiar Nuclear Element in the Male Reproductive Cells of Insects. Zoöl. Bull., II.

'00 The Spermatocyte Divisions of the Acrididæ. Kans. Univ. Quart., IX., 1.

'01 Notes on the Accessory Chromosome. Anat. Anz., Bd. 20, Nos. 8 and 9.

'02a The Spermatocyte Divisions of the Locustidæ. Kansas Univ. Sci. Bull., 1.

'02b The Accessory Chromosome. Sex Determinant? Biol. Bull., III., 1 and 2.

'05 The Chromosome Complex of Orthopteran Spermatocytes. Biol. Bull., 9.

McGill, C.

'04 Spermatogenesis of Anax junius. Univ. Missouri Stud., II., 5.

Medes, G.

'05 The Spermatogenesis of Scutigera forceps. Biol. Bull., 9.

Montgomery, T. H.

'98 The Spermatogenesis in Pentatoma up to the Formation of the Spermatid. Zool. Jahrb., 12.

'01a Further Studies on the Chromosomes of the Hemiptera heteroptera. Proc. Acad. Nat. Sci. Philadelphia.

'01b A Study of the Chromosomes of the Germ-cells of Metazoa. Trans. Am. Phil Soc., XX.

'04 Some Observations and Considerations on the Maturation Phenomena of the Germ-cells. Biol. Bull., VI., 3.

'05 The Spermatogenesis of Syrbula and Lycosa, with General Considerations upon Chromosome Reduction and the Heterochromosomes. Proc. Acad. Nat. Sci. Philadelphia.

'06 Chromosomes in the Spermatogenesis of the Hemiptera Heteroptera. Trans. Am. Phil. Soc., XXI., p. 97.

Morgan, T. H.

'08 The Production of Two Kinds of Spermatozoa in Phylloxerans. Functional "Female Producing" and Rudimentary Spermatozoa. Proc. Soc. Exp. Biology and Medicine, Vol. 5, No. 3.

Moore and Robinson.

'07 On the Behavior of the Nucleolus in the Spermatogenesis of Periplaneta americana. Quart. Journ. Mic. Sci., 48.

Nowlin, W. N.

'06 A Study of the Spermatogenesis of Coptocycla aurichalcea and Coptocycla guttata with Especial Reference to the Problem of Sex Determination. Journ. Exp. Zoöl., III., 4.

Paulmier, F. C.

'99 The Spermatogenesis of Anasa tristis. Journ. Morphol., Supplement to Vol. XV.

Payne, F.

'08 On the Sexual Differences of the Chromosome-groups in Galgulus oculatus. Biol. Bull., Vol. XIV., 5.

Randolph, H.

'08 On the Spermatogenesis of the Earwig, Anisolabis maritima. Biol. Bull., XV., 2.

Robertson, W. R. R.

'08 The Chromosome Complex of Syrbula admirabilis. Kansas Univ. Sci. Bull., IV., 13, Sept., 1908.

Stevens, N. M.

'05 A Study of the Germ-cells of Aphis rosæ and Aphis œnotheræ. Journ. Exp. Zoöl., II., 3.

'05a Studies in Spermatogenesis with Especial Reference to the "Accessory Chromosome." Publication No. 36, Carnegie Institution of Washington.

'06 Studies on the Germ-cells of Aphids. Publication No. 51, Carnegie Institution of Washington.

'06a Studies in Spermatogenesis, II. A Comparative Study of the Heterochromosomes in Certain Species of Coleoptera, Hemiptera and Lepidoptera, with Especial Reference to Sex Determination. Carnegie Inst., Washington, Pub. 36, No. 2.

'08 A Study of the Germ-cells of Certain Diptera, with Reference to the Heterochromosomes and the Phenomena of Synapsis. Journ. Exp. Zoöl., V., 3.

'08a The Chromosomes in Diabrotica vittata, Diabrotica soror and Diabrotica 12-punctata. Journ. Exp. Zoöl., V., 4.

'09a Further Studies on the Chromosomes of the Coleoptera. Journ. Exp. Zoöl., VI., 1.

'09b An Unpaired Heterochromosome in the Aphids. Ibid.

Sinety, R. de.

'01 Recherches sur la Biologie et l'Anatomie des Phasmes. La Cellule, XIX., p. 117.

Sutton, W. S.

'00 Spermatogonial Divisions in Brachystola magna. Kans. Univ. Quart., IX., p. 135.

'02 On the Morphology of the Chromosome-group in Brachystola magna. Biol. Bull., IV., 1.

'03 The Chromosomes in Heredity. Biol. Bull., IV., 5.

Voinov, D. M.

'04 Sur une Disposition speciale de la Chromatine, dans la Spermatogenese du Gryllus campestris, Reproduisant des Structures Observees seulement dans l'Ovogenese. Arch. d. Zool. Exp. et Genl., 4, Series 2, Notes et Revues, p. 63.

Wallace, L. B.

'00 The Accessory Chromosome in the Spider. Anat. Anz., 18.

'05 The Spermatogenesis of the Spider. Biol. Bull., VIII., 3.

Wassilieff, A.

'07 Die Spermatogenese von Blatta germanica. Arch. Mik. Anat., LXX.

Wilcox, E. V.

'95 Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen. Bull. Mus. Comp. Zoöl. Harvard Univ., 27.

'96 Further Studies on the Spermatogenesis of Caloptenus femur-rubrum. Ibid., 29.

Wilson, E. B.

'05 Studies on Chromosomes, I. The Behavior of the Idiochromosomes in Hemiptera. Journ. Exp. Zoöl., II., 3.

'05a Studies on Chromosomes, II. The Paired Microchromosomes, Idiochromosomes and Heterotropic Chromosomes in Hemiptera. Journ. Exp. Zoöl., II., 4.

'05b The Chromosomes in Relation to the Determination of Sex in Insects. Science, XXII., 564, Oct. 20, 1905.

'06 The Sexual Differences of the Chromosome-groups in Hemiptera, with some Considerations on the Determination of Sex. Journ. Exp. Zoöl., III., 1.

'07 Note on the Chromosome groups of Metapodius and Banasa. Biol. Bull., XII., 5.

'07a The Case of Anasa tristis. Science, N. S., Vol. 25, Feb., 1907.

'09a The "Accessory" Chromosome in Syromastes and Pyrrhocoris with a Comparative Review of the Types of Sexual Differences of the Chromosome-groups. Journ. Exp. Zoöl., VI., 1.

'09b Studies on Chromosomes, V. The Chromosomes of Metapodius. A Contribution to the Hypothesis of the Genetic Continuity of Chromosomes. Journ. Exp. Zoöl., VI., in press.

Zweiger, H.

'06 Die Spermatogenese von Forficula auricularia. Zool. Anz., 30, p. 220.

THE HYO-BRANCHIAL APPARATUS OF TYPHLOTRITON SPELÆUS STEJN.

WILLIAM A. HILTON, PH.D.

The cave salamander *Typhlotriton* was put in the family Desmognathidæ by Stejneger, in 1892.[1] The basis of this classification was chiefly certain skeletal characters, such as the structure of the vertebræ. The study of the hyo-branchial apparatus of this form seems to show other relationships.

In the larvæ of *Typhlotriton,* which are often found almost as large as the adult, only three branchial arches are found, Fig. 1, instead of four which are found in the larvæ of other members of the family Desmognathidæ, Fig. 2.

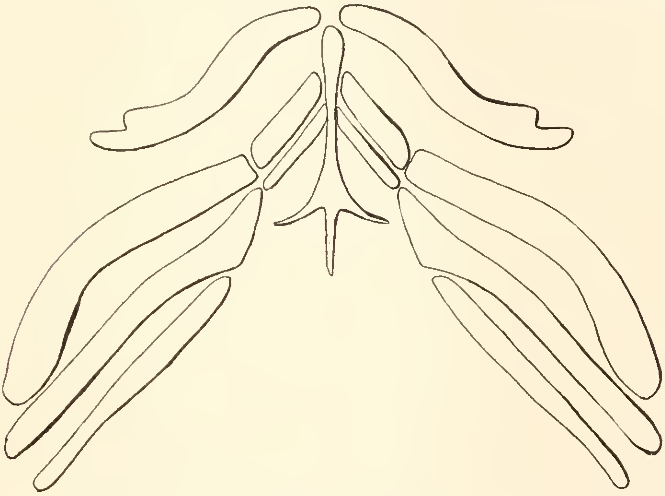


FIG. 1. Hyo-branchial apparatus of full grown larva of *Typhlotriton spelæus* from above, showing three branchial bars and a three-pointed "Copulastiel." × 6.

In the adult *Typhlotriton,* the general character of the hyo-branchial apparatus is much like the type found commonly in the family Plethodontidæ, especially in the character of the very long first cerato-branchial, Fig. 3.

---

[1] Stejneger, Leonhard, "Preliminary Description of a New Genus and Species of Blind Cave Salamander from North America, *Proc. U. S. Nat. Museum,* Vol. XV., 1892.

By a comparison of the hyo-branchial apparatus of larval *Typhlotriton* with the same parts in the larvæ of the Plethodontidæ, a striking resemblance may be noticed, especially in the fact that in *Typhlotriton* as in *Spelerpes* for instance, Fig. 4, there are only three branchial bars, while the general proportions of all the parts are about the same, as the figures show.

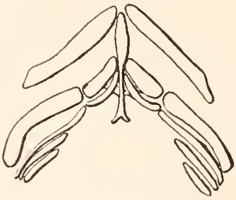


FIG. 2. Hyo-branchial apparatus of nearly full grown larva of *Desmognathus fusca* from above, showing four branchial bars. × 6.

If the hyoid apparatus of *Typhlotriton* larva be compared with that of *Typhlomolge,* another cave form, but one that has external gills, a very striking resemblance is found between the two, Fig. 5. Emerson[1] who describes the hyo-branchial apparatus of this form, suggests that it shows in many ways a marked resemblance to a *Spelerpes* larva and that it differs widely from the members of the family Proteidæ in which it has been placed.

May not this then be a larva, possibly a permanent larva as suggested by Kingsbury[2] for *Necturus?* At any rate judging from the hyoid apparatus alone there seems to be a rather close relationship between the two forms *Typhlomolge* and *Typhlotriton.*

In comparing the hyoid apparatus of *Typhlotriton* with that of larval *Spelerpes,* it may be noted that in all essential respects the two structures are alike. There is a slight difference in the proportions of parts, and the tip of the " Copulastiel " of Gaup, has three parts in *Typhlotriton* while it only has one in *Spelerpes.* The similar one of *Typhlomolge* we find divided into two.

---

[1] Emerson, 2d, Ellen T., " General Anatomy of *Typhlomolge rathbuni,*" *Proc. Bost. Soc. Nat. Hist.,* Vol. 32, No. 3.

[2] Kingsbury, B. F., " The Rank of *Necturus* among Tailed Bacteria," BIOL. BULL., Vol. VIII., 1905.

A summary of the points in which *Typhlomolge, Typhlotriton* and *Spelerpes* agree would be somewhat as follows:

1. All have three branchial bars in the larval form. The so-called adult, but possible larva *Typhlomolge,* has three.

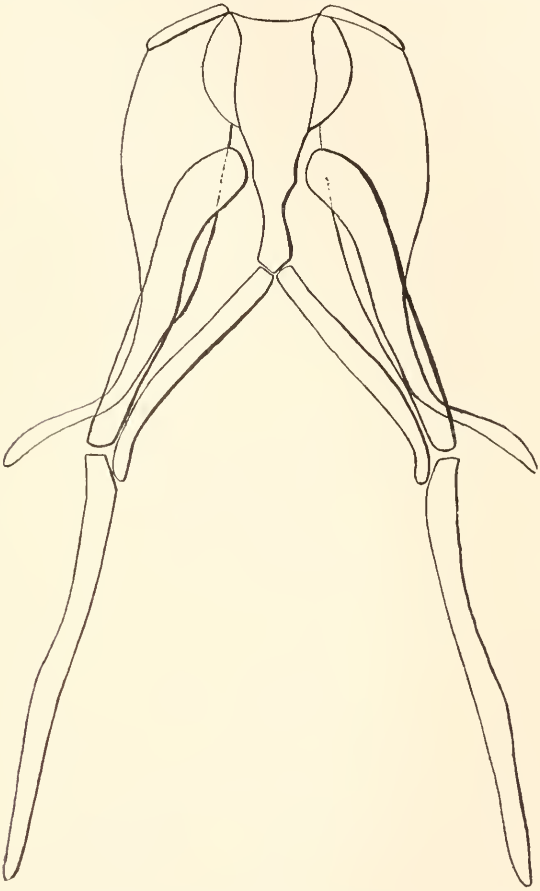


Fig. 3. Hyo-branchial apparatus of adult *Typhlotriton* from below, showing the long projecting 1st cerato-branchial. × 6.

2. The proportions and general position of the parts of the hyo-branchial apparatus are much the same in all.

3. In the adult forms of *Typhlotriton* and *Spelerpes,* the first cerato-branchial is very long.

4. The larvæ of *Typhlotriton* and *Spelerpes* grow to some size before transforming.

5. All forms live away from the light much or all of the time, two have lost the use of their eyes and numbers of the genus *Spelerpes* live in caves to some degree.

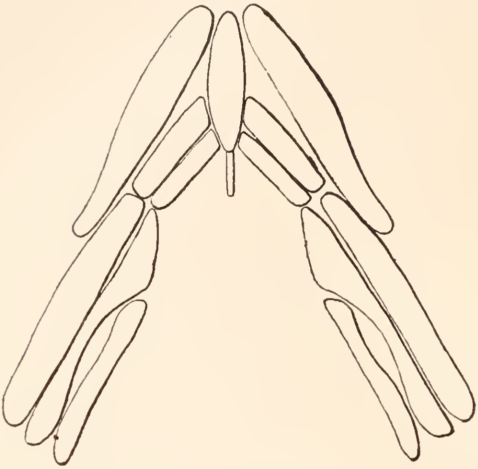


FIG. 4. Hyo-branchial apparatus of full-grown larvæ of *Spelerpes bilineatus* from below, showing the single "Copulastiel." × 6.

Judging from these similarities may not a series of forms be named in which all the members are closely related to each other, and which shows the different degrees of adaptation of one distinct line of Urodela?

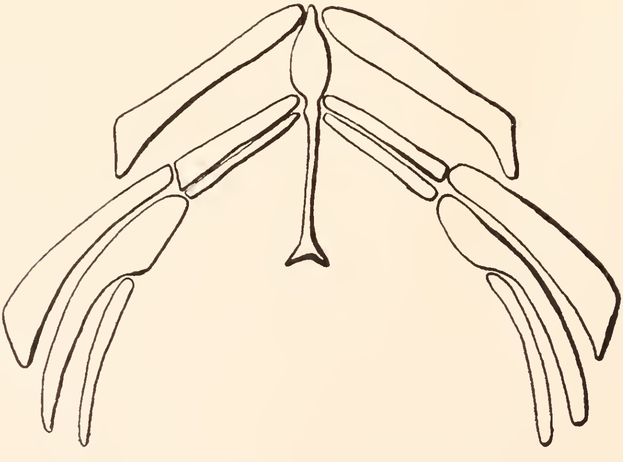


FIG. 5. Hyo-branchial apparatus of *Typhlomolge rathbuni* from above, after Emerson. × 6.

1. *Spelerpes bilineatus* which is not found in caves to any degree.

2. *Spelerpes longicauda* resembling the first form, but often called a cave salamander, although it may be found out from caves now and then.

3. *Spelerpes maculicaudus.* More properly a cave form and closely resembles the next form in many ways on the one hand, as well as *longicauda* on the other, but has well developed eyes.

4. *Typhlotriton*, found in caves. Has lost the use of its eyes and is truly a cave form.

5. *Typhlomolge rathbuni* which is found in deeper caves and seems to be a permanent larva or the larva of an adult closely related to *Typhlotriton* and *Spelerpes*.

Laboratory of Histology and Embryology,
Cornell University, Ithaca, N. Y.

THE AUTOTOMY OF THE HYDRANTH OF TUBULARIA.

MAX MORSE.

When colonies of *Tubularia crocea* are brought into the laboratory, and placed in aquaria, they sooner or later lose their hydranths. The hydranths become pinched off from the stems and fall to the bottom of the aquaria where they disintegrate. Later, new hydranths are regenerated in place of the old ones.

Somewhat similar cases of the loss of the hydranth have been reported by various workers on other species. All such instances, however, involve an absorption of the hydranth, preceded by a dissolution of the body through histolysis. *Tubularia,* alone, is known to pinch off its hydranths completely from the stems before extensive histolysis occurs or indeed, any whatsoever.

Loeb[1] found in a species of *Campanularia,* an absorption of the hydranth when the individual came into contact with the sides of the dish or with any other solid object. Thacher[2] corroborated Loeb so far as an absorption occurred, but no evidence of any effect of contact in producing the action could be determined. Loeb also reported absorption in two other genera, viz., *Margelis* and *Antennularia,* where, however, the inciting cause was believed by Loeb to be the changes the stems underwent under the action of gravity. No one has studied this special feature in these forms since Loeb. As will be spoken of later, *Tubularia* does not show any such response.

Thacher (*l. c.*) described absorption in two species of *Eudendrium, E. ramosum* and *E. tenue,* and also in *Pennaria tiarella. Pennaria carolinii,* an allied species, was studied by

---

[1] Loeb, J., "The Transformation and Regeneration of Organs," *Amer. Journ. Phys.,* Vol. 4, 1900.

[2] Thacher, H. F., "Absorption of the Hydranth in Hydroid Polyps," BIOL. BULL., Vol. 5, p. 297.

Cerfontaine[1] and by Gast and Godlewski.[2] Cerfontaine believed that the cause of the absorption lay in the difficulty with which the fully formed hydranth responded to sudden changes in its environment, whereas the regenerated ones are better adapted by " une acclimation rapide."

Gast and Godlewski (*l. c.*) have examined the case more critically. They show that, at the beginning of the process, the tentacles are withdrawn until nothing is left externally to show where they once existed. The peristome likewise disappears and the mouth closes. Ultimately, all that is left of the hydranth is a small smooth knob. This, too, becomes absorbed into the stem and a cicatrix alone remains. Then regeneration may begin. Concomitant with these external changes, the cells of the endoderm begin to dissociate and disintegrate and come to lie as detritus in the lumen of the hydranth. The current flows down into the stem and carries the débris and this continues until the whole of the hydranth has been absorbed. The changes in the cells are not restricted to the endoderm cells for those of the ectoderm lose their vacuoles and become greatly reduced in size. They remain, however, much longer *in situ* than the cells of the endoderm. The changes seem to affect, from the first, all of the cells of the hydranth and the process is only in this sense a local one. Moreover, inasmuch as open communication between the hydranth and the rest of the stem is maintained, it cannot be urged that the process is a passive one, due simply to the death of the cells of the hydranth. In this respect, the description of Gast and Godlewski is similar to that to be given for *Tubularia*.

The first evidence of autotomy in *Tubularia* is the sinking of the hydranth upon the stem. This pendent position results from a constriction of the cœnosarc at the base of the hydranth, where it joins the stem. For a time the hydranth hangs suspended by the perisarc, but soon this breaks and the hydranth falls. During the whole of this process, including, generally, the first half hour while the hydranth lies at the bottom of the dish, completely severed from the stem, the tentacles are seen to move about as in a normal individual. After a longer or shorter time, varying with external conditions, the movements cease, the hydranth becomes whitish and evidences of disintegration become apparent. The cœnosarc of the stem, on being freed from the hydranth, early shows a collection of red pigment, described by Driesch, Loeb, Stevens and Morgan in their studies of regeneration in this genus. The tube of the stem has been closed distally by the folding and compression of the walls even before the hydranth has been lost. For a time immediately after the autotomy of the hydranth has taken place, the cœnosarc withdraws for one of two millimeters down the perisarc tube, leaving free, ragged edges to the outer tube projecting beyond the protoplasmic portions. Soon, however, the cœnosarc is seen to be flush with the top of the perisarc.

---

[1] Cerfontaine, P., " Recherches experimentale sur la regeneration et l'heteromorphose chez astroides calycularis et *Pennaria carolinii*," *Arch. de Biol.*, t. 19, 1902.

[2] Gast, R., und Godlewski, E., Jr., " Über den Regulationserscheinungen bei *Pennaria carolinii*," *Arch. für Entwickelungsmec.*, Bd. 16, 1903.

Sections through hydranths and stems at the period when the hydranth is falling, show a constriction of the protoplasmic parts immediately beneath the attachment of the hydranth to the stem. The perisarc seems to take no active part in the changes involved in autotomy. The walls of the cœnosarc, involving both ectoderm and entoderm, are folded upon themselves. In some specimens there seems to be an indication of histolysis setting in among the cells forming the ectoderm and entoderm in the portion of the cœnosarc tube involved in the constriction. When it occurs, such dissociation of the cells is of local occurrence only and is to be found only in the constriction. Examination of the hydranth in such cases (Fig. 1), shows it to be perfectly normal as far as one may judge from histological evidence. Moreover, living specimens, as has been mentioned, show activity of the tentacles even after the hydranth has been removed.

For a time, varying with the individual, the hydranth remains attached to the stem by the perisarc only and it is the weight, probably, of the hydranth which ruptures the chitinous perisarc and frees the hydranth from the stem. One may see, in the individuals at this stage, resting in the aquaria, that all protoplasmic attachment has been withdrawn and longitudinal sections bear this out. At the base of the hydranth and at the distal end of the stem, from which the hydranth has been severed, there is no definite arrangement of the cells into ectoderm and entoderm. The cells seem to lie freely in the chitinous envelop. The cylindrical or flattened epithelium cells of ectoderm and entoderm of the normal individual have now assumed a spherical form. In the iron-alum stain, the nuclei appear for a time normal, but later distinct changes, undoubtedly pathological, have set in. There is no indication of an absorption of such cells into the lower portions of the stems as Gast and Godlewski (*l. c.*) have described for *Pennaria.*

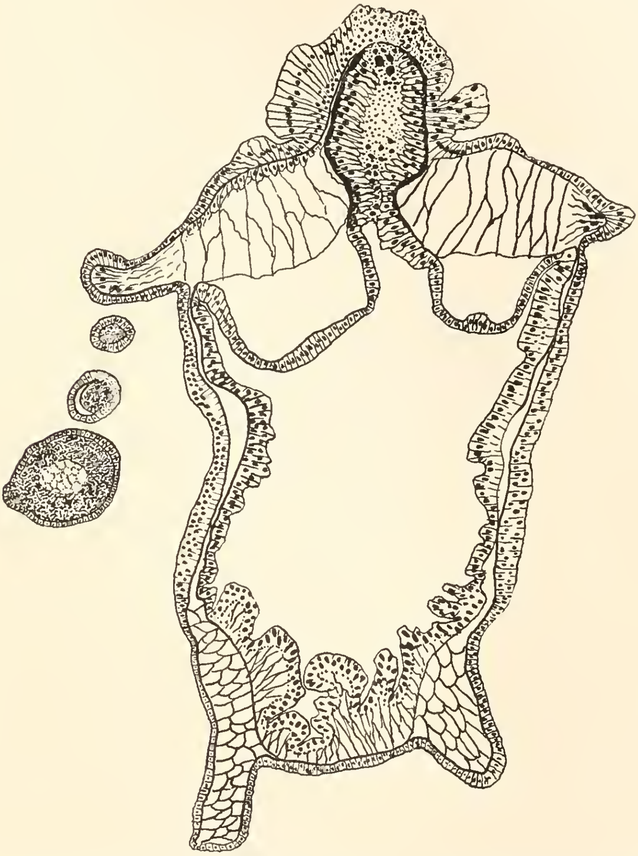


FIG. 1. Sagittal section of *Tubularia* hydranth immediately after autotomy. The hydranth had fallen to the bottom of the dish.

The process of disintegration of the cells of the hydranth which has been cut off from the stem, is ushered in by dissociation of the cells of the entoderm (Fig. 2). There is no constancy in location of the origin of this process, but, in the main, it seems to set in at the proximal end, *i. e.,* at the end formerly connected with the stem. The entoderm cells leave their positions around the periphery of the hydranth, immediately beneath the ectoderm and come to fill the whole of the cavity of the hydranth. This cavity, at the same time, begins to become smaller in volume. Then, after a time, the ectoderm cells begin to participate in the process of dissolution. The gonanths are the last to suffer (see Fig. 2). Even after the hydranth has begun to lose its shape, as it lies at the bottom of the dish, these reproductive bodies show little histolysis.

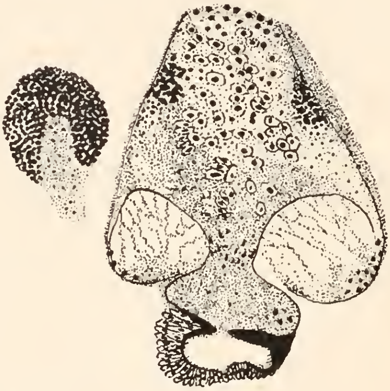


FIG. 2. Hydranth which has begun to disintegrate, in sagittal section. A gonanth is shown also in section; the cells are normal.

In order to determine what factors were operative in causing autotomy, the writer performed a series of experiments on *Tubularia.* The following external factors were considered: (1) Temperature effects—(*a*) heat, (*b*) cold; (2) light, involving its absence, darkness; (3) gravity; (4) aëration; (5) mechanical factors, such as abrasion, force of current, etc.

1. *Temperature.* (*a*) *Effects of Heat.*—A fingerbowl, containing a dozen stems of *Tubularia* fresh from the sea, was placed in the direct sunlight, where the temperature registered

25° C. A control was placed in the shadow near it.[1] Within half an hour evidences of autotomy became apparent and within one and one half hours, over half of the hydranths had been lost. In the control, where the temperature of the water registered 5 degrees lower (25° C.), the hydranths were retained for half a day. More striking still was the rapidity of autotomy in the case of the fingerbowl of specimens placed in an apartment immediately over the boilers of the engine-room of the Bureau of Fisheries Laboratory where the temperature is constantly at 33° C. In this case all of the hydranths had fallen entirely from the stems within two hours, while a few had fallen within one hour.

(*b*) *Cold Effects.*—A fingerbowl of specimens, 12 in number, was placed in an ice-chest at a temperature of 8° C. The hydranths in this case were retained for three weeks and undoubtedly would have persisted had not the experiment been terminated accidentally. The specimens were examined from day to day and they gave every indication of being normal, inasmuch as the tentacles were active at all times. Food was provided for the hydroids, but it was not possible to determine whether they fed or not. In another set of experiments, specimens were kept a week at a temperature of 10° C. in a fingerbowl resting in chipped ice, the bowl being placed in diffuse light. At the end of the week, the individuals were apparently healthy and no indication of autotomy could be determined.

It cannot be urged against these experiments that the animals became benumbed and their activities thereby lessened with consequent retention of the hydranths, for as we have seen, they were aparently normal and active throughout.

2. *Effect of Sunlight.*—In the experiments with the effects of light, it was necessary to insure against heat effects. Various methods were used for obviating this factor. A large vessel, with a volume of about six gallons, was provided with a platform which rested about ten centimeters from the bottom of the vessel and fifteen from the surface of the water over it. On this platform, a fingerbowl of *Tubularia* stems was placed. Then

---

[1] The possibility of light being the factor here is considered later.

the larger vessel was filled with water so that the fingerbowl was submerged to a depth of fifteen centimeters. The apparatus was placed in the direct sunlight, which fell upon it from eight o'clock in the morning until about four thirty in the afternoon. The temperature of the water was kept at 20° C. It was not possible to reduce the temperature of the water, under the circumstances, below this. Control specimens were placed in a similar vessel, somewhat smaller, in the shadow near the larger one. The first hydranths fell from the individuals in the larger vessel, exposed to the rays of the sun, during the third day. Those in the control gave evidence of autotomy at about the same time, or even earlier, perhaps. This result is quite similar to that given by colonies of *Tubularia* brought into the laboratory and placed in running salt water either in the light or in the dark. It seems to point to the absence of any effect of sunlight in inducing the process of autotomy. The hydranths very probably would have remained longer if the temperature could have been reduced, as is shown in other experiments to be described.

In another set of experiments fingerbowls of specimens were placed in a trough of chipped ice and set in the direct sunlight. The hydranths were retained for nearly two weeks. In a third set, under more natural conditions, stems of the hydroid were tied to weighted blocks of wood and these were submerged about fifteen centimeters beneath the surface of the water in the "outer pool" of the Bureau of Fisheries at Woods Hole, where the tide sweeps through at all times, bringing cool water from Vineyard Sound and Buzzards Bay. The individuals, of course, were subjected to the direct rays of the sun. The hydranths were retained for two weeks and this would have continued undoubtedly if the waves during a stormy period had not torn the specimens from their supports. The average daily temperature of the surface water during this time of year was 19° C. At night the temperature fell to 15° C.

We may conclude from these experiments that sunlight is not a factor in producing autotomy in *Tubularia*.

With respect to darkness, data have been given already to show that this is not a potent factor. In the case of hydranths placed in the ice-chest, the individuals were not exposed to light for more than a few moments during the day. Therefore, if the absence of illumination was a positive factor in inducing the process of autotomy, it most assuredly would have been made evident here. In another case, specimens were attached to weighted blocks of wood, as used in an earlier experiment, the block being covered with a tin case. These were lowered beneath a bridge over a tide-way to a depth of two fathoms. The individuals were therefore in almost total darkness. The temperature of the water at this depth was lower than that of the surface, owing to tide currents. The hydranths were retained for over two weeks, when the experiment was terminated.

3. Stems of *Tubularia* were tied to supports in running water of a temperature of about 20° C. The supports could be so arranged that the stems were inclined at any angle with respect to gravity. The hydranths were cut off by autotomy in three days at whatever angle the stems were placed. Inasmuch as this time corresponds to that in which the hydroids of colonies introduced into aquaria from the sea, lose their hydranths and since no decrease in the time of autotomy could be determined, to be correlated with the position of the hydroid with respect to gravity, the writer does not believe that this factor is operative in autotomy. Loeb, it will be recalled (*l. c.*), believed that gravity induced absorption in *Antennularia.* This case of Loeb's is the only one recorded in which gravity acts. When one recalls the fact that the stems of *Tubularia* are placed in any position with respect to the vertical in their natural habitat on rocks and piles, it would seem quite extraordinary that such effects of gravity should obtain in this species. Moreover, in strong currents, such as the tide which sweeps over the rocks of the Tidemill at South Harpswell, Maine, the stems are subjected to a force which causes incessant change in their axes with respect to gravity.

4. To determine the effect of lack of oxygen, a liter of sea water was boiled for half an hour and cooled to 20° C. in an hermetically sealed vessel. Specimens of *Tubularia* were put into the water and the vessel, now exposed to the air, was placed in the ice-chest. Within two hours nearly every hydranth had fallen from the stems.

In another set of experiments, excess of oxygen was supplied by means of the Mast aërating device. The vessel containing the hydroid stems was placed in chipped ice and the aërating apparatus delivered bubbles of air to one corner of the dish. However, here, no lengthening of the period of retention of the hydranths could be determined when the temperature was permitted to rise to ordinary room temperature (21° C.). Therefore, while lack of oxygen causes autotomy to set in, an excess of the gas over and above that which forms normally the oxygen content of sea water at these temperatures does not prolong the period of retention of the hydranths; they behave as if they had been brought in from the sea and placed in an ordinary aquarium under ordinary conditions.

It will be recalled that the specimens in the fingerbowls which were kept in the ice-chest were not supplied with air artificially. Moreover, the water was not changed, so that the air which was over the surface of the water of the bowls was sufficient to supply the oxygen needed by the animals. The oxygen content of water kept under such conditions cannot vary to any great degree and the sea water, flowing over the animals in their natural habitats varies, as has been determined, comparatively little in the amount of air in solution. Moreover, the specimens which, on being introduced into the laboratory lose their hydranths, are kept, in the main, supplied with ever changing water in which the oxygen content is practically that of the surrounding sea, or even higher, owing to the condensation by the pumps. Therefore this factor cannot be of any considerable importance in causing autotomy.

5. Considerations were given to mechanical effects such as the swaying of hydranths on the stems in a strong current of water, rubbing of the stems against one another and mechanical shock induced by cutting off the hydranths from the stems.

With respect to the first factor, we know that in nature, in many cases, *Tubularia* grows where tide causes whirlpools and an ever changing direction in the currents passing over the hydroids and therefore, one would not look for this as a cause of autotomy. Experiments were performed to show that the factor is not operative, by placing stems, bearing healthy hydranths in jets of water which caused an incessant waving of the hydranth on the stem. The hydranths were retained, under such conditions, as long as was compatible with the temperature and other external conditions. Abrasion was likewise made the question of another set of experiments and here, too, no effect could be correlated with the factor. With respect to mechanical shock, when the stems are removed from the colonies, data have already been given, for in the ice-chest experiments, where the hydranths were retained for a long time, the stems had been cut from the colony. Moreover, in those specimens which were placed on floats in the sea and where the hydranths did not fall off, the individuals had been cut from the stems. Therefore, mere cutting of the stems is not a factor in autotomy.

It will be seen that temperature seems to be the only consistent factor involved in the decapitation. When the temperature is kept at about 10° C. or 15° C., the hydranths are retained, regardless of any other factors with the one exception of lack of oxygen, which we believe to be inoperative except under wholly artificial conditions. When it is recalled that in the sea, *Tubularia* may be found throughout the summer[1] in latitudes where the temperature of the water does not rise above 18° C. or 20° C., while in lower latitudes[2] the occurrence of this species on the piles and rocks is correlated with the times of the year, spring and fall, when the water is cooler, we find sufficient data from natural sources to conclude that temperature is the potent factor in causing autotomy of the hydranths in colonies brought into the laboratory.

The case of *Hydra,* described by Greely[3] where the body becomes reduced to a mass of dissociated cells when the temperature is reduced to 4° C. and 6° C., seems to weigh against the present conclusions, but more recently Caroline McGill[4] has studied the histological changes occurring in *Hydra* subjected to reduced temperatures and is unable to corroborate Greely, but rather shows that a loss of water accompanies the lowering of the temperature, which causes the hydroids to become opaque, without degenerative changes in the cells.

---

[1] As at South Harpswell, Maine, where the temperature of the water seldom rises above 16° C.

[2] *E. g.*, Woods Hole, Mass. *Tubularia* disappears when the temperature of the water reaches 20° C. It reappears again for a time in the fall.

[3] Greely, "Further Studies on the Effects of Variations of Temperature on Animal Tissues," Scientific Papers.

[4] McGill, C., "The Effect of Low Temperature on *Hydra*," BIOL. BULL., Vol. 14, 78.

Little success has been had with *Tubularia* in exhibition tanks. If some means could be devised for keeping the water in such tanks at a temperature, say of 15° C., the writer believes that *Tubularia* could be maintained in health, as in the sea.

The writer has studied *Tubularia* at the Bureau of Fisheries and the Marine Biological Laboratory, at Woods Hole and at the Harpswell Laboratory. To those in charge of these laboratories, Professors Sumner, Lillie and Neal and to Dr. Kingsley and Professor Lambert, the writer is indebted for much kindness. The work was started at the suggestion of Dr. T. H. Morgan and the writer is grateful to him for generous aid and criticism.

College of the City of New York,
December 15, 1908.

PRELIMINARY REPORT ON THE EARLY HISTORY OF THE EGG AND EMBRYO OF CERTAIN HYDROIDS.

CORA JIPSON BECKWITH.

Within the last few years some of the hydroids (*Pennaria, Clava leptostyla, Eudendrium, Tubularia crocea*) have been described as differing widely in their processes of maturation, fertilization, and early cleavage from what we have come to think of as typical. Other members of the same group (*Tubularia mesembryanthcum, Clava squamata, Hydra, Gonothyrea, Æquorea, Tiara, Gonionemus*) have been described as perfectly typical. That certain of the hydroids should conform to the type and others not, seemed improbable. The fact of the low organization of the group which is used by Hargitt to account for such variation seemed hardly sufficient. On this account at the suggestion of Professors Morgan and Wilson, I undertook the reëxamination of two of the forms, *Pennaria* and *Clava leptostyla*, which varied most from the type, to discover if possible the relation between the aberrations described in these forms and the usual type. The results on *Pennaria* were worked out in the winter of 1907 at the Zoölogical Laboratory of Columbia University, on material collected at Woods Hole during the previous summer. Those on *Clava* were obtained in the summer and autumn of 1908.

A brief review of Hargitt's work on *Pennaria* and *Clava leptostyla* will be necessary to recall the points which I wished to clear up. In both these forms, according to his results, the processes of maturation and fertilization of the egg are very obscure and incapable of demonstration. The germinal vesicle just before the time that maturation should take place moves to the periphery of the egg where it loses its staining capacity, the nuclear membrane breaks down, and the nuclear substance becomes diffused throughout the egg, where it is no longer recognizable, due probably to some chemical or physical change in the egg. The maturation and fertilization processes were supposed to take place while the chromatin is in this unstaining condition, the sperm having also lost its staining capacity after entering the egg. The first evidence of nuclei in the egg after the disappearance of the germinal vesicle was found in the appearance of "nuclear nests" or groups of small vesicles scattered throughout the egg, several such nests usually occurring in an unsegmented egg. In *Pennaria* not less than four such nests appear simultaneously indicating four centers of nuclear reconstruction. In *Clava*, as I understand the description, this condition of several nuclear groups occurs occasionally in an unsegmented egg. More often, however, eggs were found already segmented with a single resting nucleus in each cell which I take it were believed to arise as described for *Pennaria*. This reappearance Hargitt thinks is explicable on the ground that after fertilization the chemical or physical conditions again change so that the chromatin once more responds to stains. The chromatic material that was scattered at the time the nucleus disappeared collects again, forms vesicles which especially in *Pennaria* occur in groups or nests, each nest finally fusing into a single nucleus. Thus a syncytium arises without mitosis and with no apparent evidence of maturation or fertilization having taken place in the egg. In *Pennaria* after these nuclear groups are formed, nuclear proliferation is by mitosis. In *Clava leptostyla,* however, up to the sixteen-cell stage Hargitt describes nuclear proliferation by amitosis, later cleavages being mitotic.

The points in question accordingly are: (1) The nature of maturation and fertilization processes, (2) the formation of nuclei *de novo*, and (3) the *rôle* of amitosis and mitosis in early cleavages.

Pennaria and *Clava leptostyla* were preserved at Woods Hole where Hargitt obtained his material. Also the same killing fluids and stains were used so that the question of method is eliminated. Material was killed every hour of the day and night and every half hour in the early morning hours which proved to be the most important period.

I found in both *Pennaria* and *Clava leptostyla* that in material killed between the hours of 4 and 6 A. M., it was possible to demonstrate the maturation processes and that they take place with the utmost exactness and in the typical manner, as reference to the figures will show. Fig. 1 shows the germinal vesicle of an egg of *Pennaria* in which the inner wall is breaking down, the nucleolus passing into the cytoplasm where it is lost, while the chromatin is grouping itself around the periphery of the nucleus to form the chromosomes. Figs. 2 and 11 are nearly corresponding stages of the first polar spindle in *Pennaria* and *Clava leptostyla* respectively. In both forms the chromosomes are evidently bipartite and the number is determined to be one half the somatic number. In the figure of *Pennaria* (which is in the late prophase) the spindle has not yet swung around into position. A comparison of Figs. 3 and 12 shows again practically identical conditions of the second polar spindle in the two forms. The first polar body lies outside the egg, the second polar spindle is in the late anaphase. I have found numerous intermediate stages. Figs. 4 and 13 show corresponding stages of the reconstructed egg nucleus with the polar bodies lying outside the egg.

Figs. 5 and 6 give two stages in the fertilization of *Pennaria*. The two-germ nuclei lying side by side at the periphery of the egg later move toward the center of the egg where they form the fusion nucleus at the ends of which astral radiations appear. The origin of the first cleavage spindle is not determined. For lack of proper stages the fusion of the two-germ nuclei has not been demonstrated with certainty in *Clava leptostyla*.

From this point forward the two forms differ slightly. In *Pennaria,* after the first cleavage the two nuclei are reconstructed by the formation of chromosomal vesicles as shown in Figs. 7 and 8. For some reason, possibly the rapidity of nuclear divisions, the chromosomal vesicles often fail to fuse into a single nucleus but give rise to a "nuclear nest" which subsequently gives rise directly to the chromosomes of the following cleavage figure. Fig. 9 shows two such vesicles passing on to a spindle, one vesicle already broken up into the individual chromosomes, the other still in the vesicular stage. Fig. 10 shows an equatorial view of such a group of vesicles, some of the chromosomes already forming an equatorial plate.

A shortening of the resting stage between the nuclear divisions, it seems to me, might account for Hargitt's interpretation of the "nuclear nests." The rapidity of nuclear division is accompanied by slow cytoplasmic division so that the former constantly outruns the latter, the result being that an unsegmented egg often contains several such groups of vesicles or "nuclear nests."

In *Clava* the cytoplasmic cleavage does not lag so far behind the nuclear division, but in fact keeps pace with it. For this reason it was possible as shown by Figs. 14, 15, 16, 17, 18 and 19 to demonstrate the first cleavage spindle, and the successive passage of the two-cell stage into the four-cell, eight-cell and sixteen-cell stages. The nuclear reconstruction takes place here again by the formation of chromosomal vesicles; but *Clava* differs from *Pennaria* in that, as a rule, the vesicles all fuse into a single nucleus between successive cleavages So far as I am able to tell as yet, it seems probable that the cleavage in *Clava* is fairly regular.

SUMMARY.

In the two hydroids (*Pennaria* and *Clava leptostyla*) under question, the maturation and fertilization processes take place in a perfectly typical fashion and form no exception to the general rule in this regard.

The conclusion that the "nuclear nests" indicate the formation of nuclei *de novo* is shown to be untenable. The occurrence of these nests is explained by the conditions of nuclear reconstruction after cleavage, the chromosomal vesicles failing to fuse between successive divisions in *Pennaria* and the cytoplasmic division lagging behind nuclear division gives a syncytium with several nuclear groups.

Maturation and the early cleavages take place by means of mitosis and not amitosis. No evidence whatever of amitotic division was found.

My results regarding the maturation and fertilization phenomena make it very probable that Hargitt's failure to observe these stages was due simply to the fact that the eggs were not obtained at the right time of day. In eggs collected at the proper time (4–6 A. M.) there is no difficulty in proving the typical stages of maturation and fertilization.

VASSAR COLLEGE,
POUGHKEEPSIE, N. Y.

BIBLIOGRAPHY.

Allen, Carrie M.

'00 The Development of Parypha crocea. Biol. Bull., Vol. I.

Bigelow, H. B.

'07 Studies on the Nuclear Cycle of Gonionemus Murbachii. Bull. of Museum of Comp. Zoöl. at Harvard College, Vol. XLVIII., No. 4.

Boveri, T.

'90 Zellen Studien (Tiara), Ueber das Verhalted der Chromatischen Kernsubstanz bei der Bildung der Richtungskörper bei der Befruchtung. Jena Zeitschr., Bd. 24, Heft 3.

Brauer, A.

'91 Über die Entwicklung von Hydra. Zeitschr. f. Wiss. Zool., LII.

'91 Ueber die Entstehung der Geschlechtsproducte und die Entwickelung von Tubularia messembryantheum Allm. Zeitschr. f. Wiss. Zool., Bd. LII.

Haecker,

'92 Die Furchung des Eis von Æquorea. Arch. f. Mikr. Anat., Bd. 40.

Hargitt, C. W.

'04 The Early Development of Pennaria. Arch. f. Entwickls. Mechan. der Organismen, Bd. XVIII.

'04 The Early Development of Eudendrium. Zool. Jahrb., Bd. XX.

'06 Development of Clava leptostyla. Biol. Bull., Vol. X.

Harm, Karl.

'02 Entwicklungsgeschichte von Clava squamata. Zeitschr. f. Wiss. Zool., Bd. LXXIII., Heft 1.

Morningstein, P.

'01 Untersuchungen über die Entwickelung von Cordylophora lacustris Allm. Zeitschr. f. Wiss. Zool., Bd. LXX.

Wulfert, J.

'02 Die Embryonal Entwicklung von Gonothyrea loveni Allm. Zeitschr. f. Wiss. Zool., Bd. LXXI., Heft. 2.

EXPLANATION OF PLATE I.

Pennaria. All drawings × 1,300.

FIG. 1. Early prophase of germinal vesicle of egg of *Pennaria;* the inner nuclear wall is breaking down; the nucleolus passing into the cytoplasm; the chromosomes forming.

FIG. 2. Later prophase of first polar spindle of egg of *Pennaria;* some of the chromosomes bipartite; some quadripartite. Reconstructed from two consecutive sections.

FIG. 3. Anaphase of second polar spindle of egg of *Pennaria.*

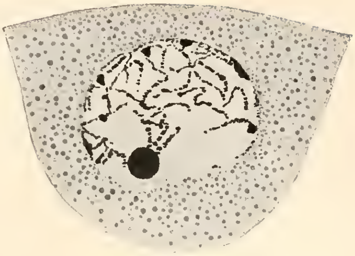
FIG. 4. Female germ nucleus and polar bodies in egg of *Pennaria;* chromatin is in fine reticulum.

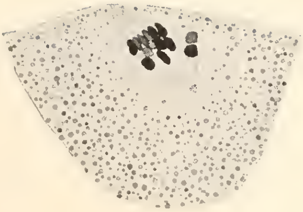
FIG. 5. Fertilization of *Pennaria* egg; male and female germ nuclei at the periphery of the egg and of equal size. Reconstructed from three consecutive sections.

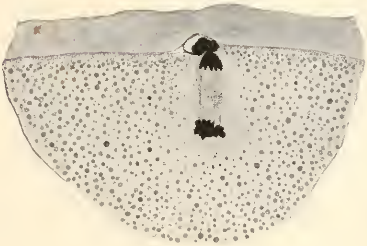
FIG. 6. Egg of *Pennaria* showing the fusion nucleus with astral radiations at either end of the nucleus.

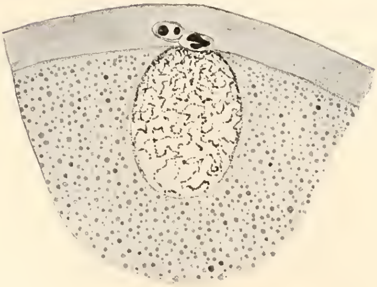
FIG. 7. Telophase of 1st cleavage of egg of *Pennaria;* nuclear reconstruction by the formation of chromosomal vesicles.

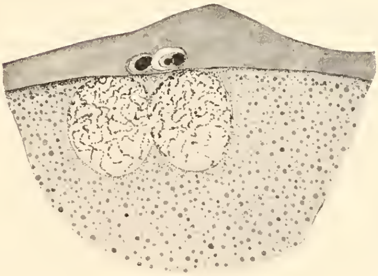
FIG. 8. "Nuclear nests" formed by the partial fusion of the chromosomal vesicles lying at the ends of the spindle (probably second or third cleavage). *Pennaria.*

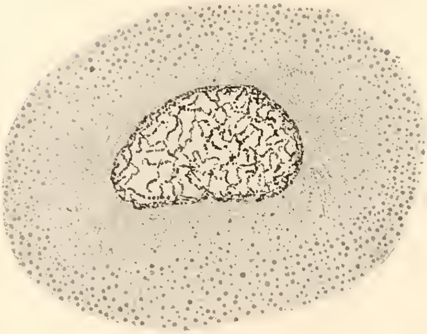

1

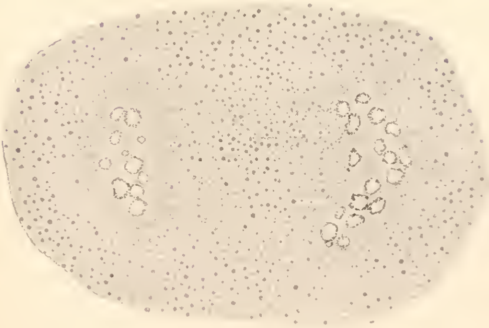

2

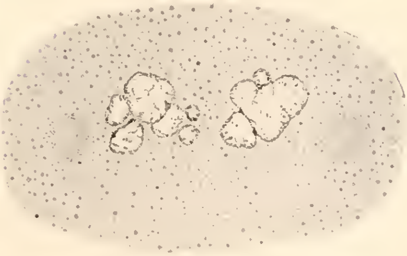

3


4


5


6


7


8

Explanation of Plate II.

Pennaria. Figs. 9 and 10, × 1,300.

Clava leptostyla. Figs. 11 to 13, × 1,300; Fig. 14, × 350.

Fig. 9. Third or fourth cleavage spindle with "nuclear nest" passing on to it to form the equatorial plate, one vesicle broken up into chromosomes. *Pennaria.*

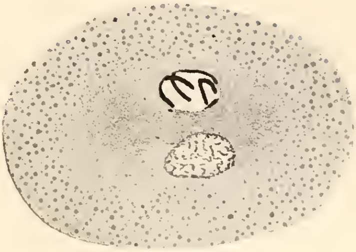
Fig. 10. Polar view of a cleavage spindle showing a "nuclear nest" as it is breaking up to form the equatorial plate, slightly later than the above, some of the chromosomes already free in the cytoplasm. *Pennaria.*

Fig. 11. Metaphase of first polar spindle of egg of *Clava leptostyla.*

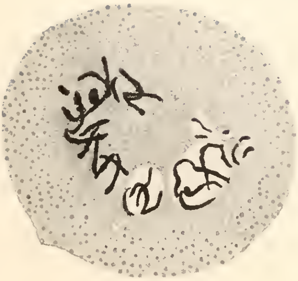
Fig. 12. Anaphase of the second polar spindle of egg of *Clava leptostyla.*

Fig. 13. Female germ nucleus and polar bodies of egg of *Clava leptostyla.*

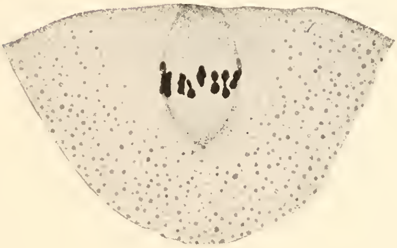
Fig. 14. Egg of *Clava leptostyla* showing the first cleavage spindle.



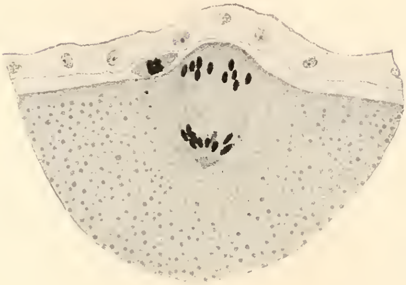
9



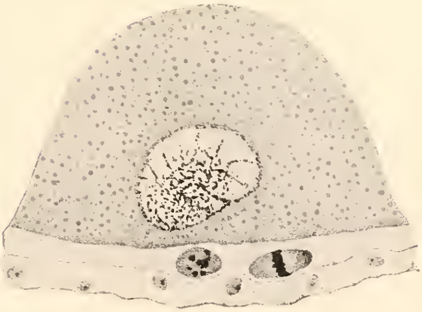
10



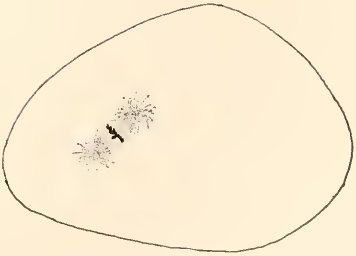
11



12



13



14

Explanation of Plate III.

Clava leptostyla. Figs. 15 to 19, × 350.

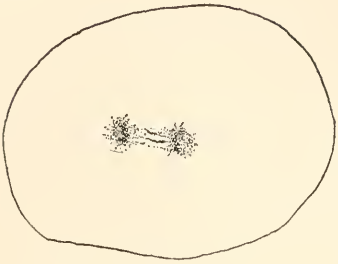
Fig. 15. Same, showing reconstruction of daughter nuclei by chromosomal vesicles.

Fig. 16. Two-cell stage of *Clava leptostyla* passing into the four-cell stage, second cleavage spindles showing.

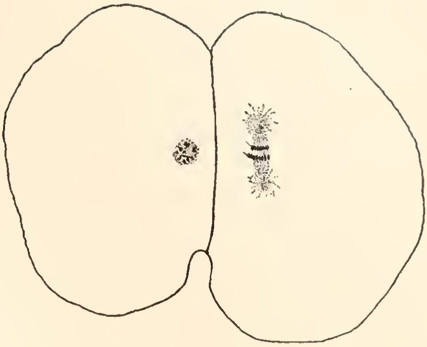
Fig. 17. Four-cell stage of *Clava leptostyla* with resting nuclei.

Fig. 18. Four-cell stage of *Clava* passing into the eight-cell stage, spindles showing in two cells.

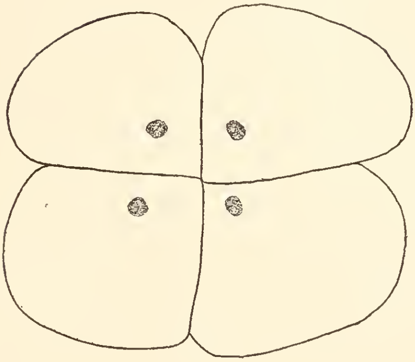
Fig. 19. Lateral view of eight-cell stage of *Clava* (five cells showing in section), some of the cells with spindles, some with resting nuclei.



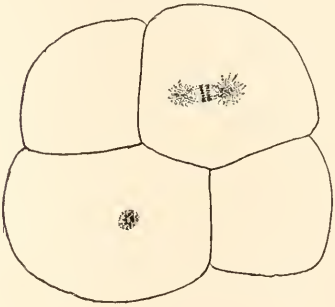
15



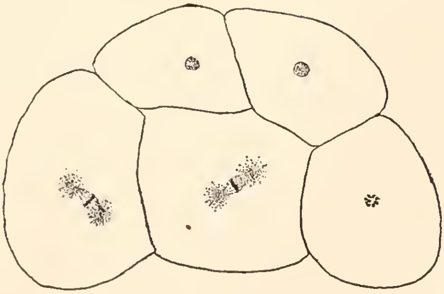
16



17



18



19

C

ON THE SEX OF HYBRID BIRDS.

MICHAEL F. GUYER,
University of Cincinnati.

In a former paper[1] I have noted the difficulty of obtaining female hybrids from pigeons or doves of widely different parentage. Of the seven hybrid offspring of very distinct species then in hand, six were male. Since that time, through the courtesy of the Museum d'Histoire Naturelle in Paris and the Museum of Natural History in London, I have had the opportunity of examining a number of different hybrids in the family Phasianidæ and among them also I have found a remarkable predominance of males. In the following tabulations the sex of each hybrid, when known, and the parentage, is given, together with the date the individual was placed in the museum. It has been impossible to give the specific name always because a number of the specimens bore only the popular names. The letters placed after the year of accession indicate the respective locations of the specimen in question; thus, B = British Museum (Museum of Natural History); P = Museum d'Histoire Naturelle, Paris; C = Museum, University of Cincinnati.

Guinea-fowl × Chicken.

	Sex.	Date and Location.
Guinea-fowl × common fowl	= ?	1902 B.
Guinea-fowl × common fowl	= ♂	1899 B.
Pintade × Poule	= ?	1854 P.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1903 C.
Black Langshang Cock × Guinea-hen	= ♂	1908 C.
Black Langshang Cock × Guinea-hen	= ♂	1909 C.

Thus, of eight guinea-chicken hybrids, the sex is known in six cases and it is invariably male.

---

[1] Guyer, M. F., "Spermatogenesis of Normal and of Hybrid Pigeons," Dissertation, University of Chicago, 1900. Also published as Bul. 22, University of Cincinnati, 1903.

Pheasant × Chicken.

Chrysolophus pictus × Bantam fowl	= ♂	1890 B.
Phasianus colchicus × Game bantam	= ♂	1902 B.
Phasianus colchicus × Spanish fowl	= ?	1845 B.
Phasianus colchicus × Common fowl	= ♂	1884 B.
Phasianus colchicus × (Japanese long-tailed cock × common hen)	= ♂	1905 B.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1845 P.
Faison × Poule	= ♂	1836 P.
Faison × Poule	= ♂	1855 P.
Faison × Poule	= ♂	1813 P.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1851 P.
Faison × Poule	= ♂	1846 P.

It will be seen that of thirteen pheasant-chicken hybrids, the twelve of which the sex is recorded are all male.

Peafowl × Chicken.

Paon × Poule Cochinchinoise	= ♂	1907 P.
Paon × Poule Cochinchinoise	= ♂	1907 P

From the foregoing it will be observed that of the total of twenty-three hybrids from markedly different parentage (guinea × chicken, pheasant × chicken, and peafowl × chicken), each one of the twenty of which the sex is known is male.

Peafowl × Peafowl.

Pavo cristatus × *Pavo muticus*	= ♂	B.

Pheasant × Pheasant.

Chrysolophus pictus × *Phasianus reevesi*	= ♂	1887 B.
Hybrid *P. colchicus-reevesi* × *Gennæus nycthemerus*	= ?	B.
Chrysolophus pictus × *Phasianus colchicus*	= ♂	1855 B.
Hybrid *P. colchicus-reevesi* × *Gennæus nycthemerus*	= ♂	1904 B.
Gennæus horsfieldi × *Phasianus versicolor*	= ♂	1866 B.
Hybrid *P. reevesi-colchicus* × *Gennæus nycthemerus*	= ♂	1902 B.
Phasianus colchicus × *Gennæus nycthemerus*	= ♂	1902 B.
Hybrid *C. pictus-amherstiæ* × *Phasianus colchicus*	= ♂	1897 B.
Chrysolophus pictus × *Gennæus nycthemerus*	= ♂	1906 B.
Phasianus colchicus × *Chrysolophus pictus*	= ♂	1904 B.
Phasianus colchicus × *Gennæus melanotus*	= ♂	1865 B.
Phasianus colchicus × *Chrysolophus amherstiæ*	= ♂	1898 B.
Lophophorus impeyanus × *Euplocamus*[1] *melanotus*	= ?	1893 P.

[1] *Euplocamus* is a synonym of *Gennæus.*

[2] Presumably *C. pictus.*

Faisan doré[2] × Faisan commun[3]	= ♂	1842 P.
Faisan à collier[4] × Faisan argenté[5]	= ?	1886 P.
Faisan commun[3] × Faisan amherst[6]	= ?	1837 P.
Faisan doré[2] × Faisan ordinaire[3]	= ♀	1853 P.
Faisan commun[3] × Faisan argenté[5]	= ♂	1837 P.
Faisan commun[3] × Faisan argenté[5]	= ♂	1843 P.
Phasianus mongolicus × *Phasianus colchicus*	= ♂	1906 B.
Phasianus colchicus × *Phasianus reevesi*	= ♀	1904 B.
Phasianus colchicus × *Phasianus torquatus*	= ♂	1894 B.
Phasianus colchicus × *Phasianus reevesi*	= ♂	1894 B.
Chrysolophus amherstiæ × *Chrysolophus pictus*	= ♂	B.
Phasianus colchicus × *Phasianus reevesi*	= ♂	1897 B.
¾ *Chrysolophus amherstiæ* × ¼ *Chrysolophus pictus*	= ♂	1887 B.
Euplocamus[1] *swinhoii* × *Euplocamus nycthemerus*	= ♂	1882 P.
Euplocamus swinhoii × *Euplocamus nycthemerus*	= ♀	1875 P.
Euplocamus lineatus × *Euplocamus nycthemerus*	= ♂	1887 P.
Euplocamus lineatus × *Euplocamus nycthemerus*	= ♀	1878 P.
Euplocamus horsfieldi × *Euplocamus lineatus*	= ♂	1819 P.
Euplocamus horsfieldi × *Euplocamus lineatus*	= ♂	1869 P.
Faisan amherst[6] × Faisan doré[2]	= ?	1882 P.
Faisan amherst × Faisan doré	= ?	1902 P.
Faisan commun[3] × Faisan à collier[4]	= ?	1860 P.
Faisan commun × Faisan à collier	= ♂	1858 P.
Faisan commun × Faisan à collier	= ♂	1843 P.

Of a total of thirty-seven hybrid pheasants, nineteen have been from parents sufficiently widely separated to be ranked by systematists as separate genera or subgenera, and of these nineteen, fifteen were of known sex, namely, fourteen males and one female. Of the remaining eighteen there were twelve males, three females and three of which the sex was undetermined.

Thus of a grand total of sixty-one hybrids, the sex is known in fifty-one cases and among these there are only four females in all. Furthermore, three of these females were hybrids between species of the same genus, the other one, between species from genera not widely divergent. In hybrids between individuals of distantly related genera or between individuals from different subfamilies (*e. g.*, guinea × chicken) where the sex has been recorded it has been invariably male.

There are three possible sources of error in these data. In the first place it is known that sterile females sometimes, although rarely, take on the male plumage, and it may be urged that there is no means of knowing certainly that the sex was determined beyond all doubt by opening the abdominal cavity and finding the testes. However, since the specimens had to be partially dissected before the skins could be mounted, it is reasonable to suppose that in the vast majority of cases the sex was thus accurately determined. The five guinea-chicken hybrids as well as the six dove and pigeon hybrids mentioned in my former paper were all dissected by me personally and consequently I am sure of their sex.

---

[3] Presumably *P. colchicus.*

[4] Presumably *P. torquatus.*

[5] Presumably *G. nycthemerus.*

[6] Presumably *C. amherstiæ.*

In the second place the objection may be raised that possibly the museums have preserved only the males, inasmuch as they make handsomer specimens and are not as similar in appearance as female pheasants. There is, of course, a possibility of this, especially in the case of hybrid pheasants from closely related species. Hybrids from widely different parents are so rare, however, that there is every probability that if there had been females they as well as the males would have been preserved. As a matter of fact, the few female pheasant hybrids that I have been able to find in museums are not similar in appearance nor do they resemble the males. As *hybrids* they are as interesting in every way as the males and it seems probable, therefore, that had there been more of them they would have been preserved. When due allowance is made for all errors the facts still indicate that there is a marked tendency for hybrids, especially those from widely separated parents, to be male.

Lastly, there is the remote possibility that there has been a greater mortality among the females in early life. In the few cases (guinea-chicken hybrids and various pigeon hybrids) of which I have data regarding the number of eggs laid and the history of the young, there is no evidence of such mortality.

It may be noted in passing that in the collections of the British Museum there is to be seen a hybrid between individuals of two different families, namely, a penelope (Family Cracidæ) and the common fowl (Family Phasianidæ). This hybrid resembles more the fowl than the penelope. Unfortunately the sex is not recorded.

In looking over the literature of the subject to see if anything had been recorded concerning the sex of hybrids outside the group Phasianidæ, I found that in general little attention had been paid to it. Some mention is made of the sex of hybrids in Suchetet's[1] voluminous work on hybrid birds. In speaking (p. cxvii) of hybrids and mongrels, he asserts that among the former he believes there are more males than females,. and he cites various authorities in substantiation of his belief. Thus, according to data collected by Buffon, there are more male than female mules and Buffon asserts, furthermore, that among hybrid birds the number of males exceeds very much that of females. Suchetet cites the following figures from Buffon: the proportion of males to females in hybrids between the he-goat and the ewe are 7 to 2; between the dog and the wolf, 3 to 1; between the goldfinch and the canary, 16 to 3. Suchetet cites still further examples from other authorities, but he seems not to have gone over his own extensive notes on hybrids with this question of sex in mind. For example, on pages cxxi–cxxxiv he gives a statement in tabular form of data collected from some eighty-five public and private museums concerning in all 234[2] specimens of hybrids between wild birds (*i. e.*, not domesticated) or of forms reputed to be such hybrids. Since in many cases the sex of these hybrids has been given, I have gone through the tables and arranged the birds according to sex as far as it is indicated, with the following results:

Of hybrids between species bearing the same generic name there are in all 124, of which 72 were male, 18 female and 34 of undetermined sex. The remaining 110 hybrids were between individuals bearing different generic names and of these 74 were male, 13 were female and 23 were of undetermined sex. Thus it will be seen that the males far outnumber the females in each case. Furthermore, this would remain true in the proportion of about 3 to 2, even should it be counted that all those of *undetermined* sex were female!

In his later amplifications of this list he discusses (p. 507) 48 additional hybrids between *Tetrao tetrix* and *Tetrao urogallus*, of which 40 are male and 8 female. Again, page 573, he lists 20 hybrids of *Lagopus albus* and *Tetrao tetrix*, of which 13 are male and seven are female.

---

[1] Suchetet, André, "Des Hybrides à L'Etat Sauvage; Oiseaux," Vol. I., 1896, Lille. A large volume of over 1,000 pages.

[2] Suchetet states the total as 236 but he has made an error of 2 in his addition on page cxxxii.

As to the general bearing of these facts upon any one of the numerous theories of sex-determination, the writer does not feel disposed to dogmatize, although certain suggestions present themselves. For a general and unbiased statement of our present knowledge regarding the question of sex-determination, the reader may consult the recent publications of Thomson[1] or of Morgan.[2]

Both of these writers agree that when all the evidence is considered it does not seem improbable that the conditions which regulate the development of sex may be different in different kinds of animals. Regarding the sex-determining influence of nutrition and temperature, either directly on the developing organism or through its parents, Thomson points out that while the evidence in any given case is inconclusive, still when all the cases are taken together, " they have a certain cumulative suggestiveness which would warrant further experiment—particularly as regards the lower animals and the indirect influence on offspring through the parents " (1908, p. 490).

In general, where the experiments tend to show that nutrition is a factor after the period of fertilization, it has been the production of females that was supposedly favored by such increased nutrition; the question being apparently one of increased constructive metabolism. It would follow that anything tending to retard or hold at a low ebb the constructive phases of metabolism, especially during early embryogeny, would be inimical to the production of females. Now in the case of hybrids, and particularly those from widely separated parents, there would in all probability be more or less default in the metabolic processes because of the incompatibilities which must necessarily exist between two germ-plasms so dissimilar. It seems not improbable, therefore, that this might be the determining factor in the production of an excess of males in the case of such hybrids.

---

[1] Thomson, J. Arthur, " Heredity," London, 1908.

[2] Morgan, T. H., " Experimental Zoology," New York and London, 1907.

THE FEMALE CHROMOSOME GROUPS IN SYROMASTES AND PYRROCHORIS.

EDMUND B. WILSON.

The conditions seen in *Syromastes marginatus* L. are of interest on account of the light that they throw on those observed by Morgan in Phylloxera, as reported by him at the December meeting of the American Society of Zoölogists, and recently published in the issue of *Science* for Feb. 5, 1909. In both forms the "accessory" chromosome is not a single but a double body, and the female chromosome-groups contain two more chromosomes than the male.

A reëxamination of the spermatogenesis of *Syromastes*[1] led to a confirmation of Gross's result that the spermatogonial number is even (22), and that the "accessory" chromosome is formed by the union of two chromosomes that are separate in the spermatogonia. This double element divides equationally in the first spermatocyte-division but passes undivided to one pole in the second, so that half the spermatozoa receive two more chromosomes (12) than the other half (10). This led me to the inference that the female somatic groups should have two more chromosomes than the male—*i. e.*, 24 instead of 22, as had been described by Gross. I had at that time no female material, but through the kindness of Professor Boveri have since obtained an abundant supply of the ovaries. Examination of this material demonstrated the correctness of my earlier inference. A considerable number of ovaries have been sectioned, many of which contain numerous and very fine division-figures, showing the chromosomes with great clearness. Whenever a good view of the equatorial plate can be obtained, 24 chromosomes are unmistakably seen to be present, as is clearly shown in photographs. Two figures (Fig. 1, *c, d*) are appended, both of which were drawn upon enlarged photographs by the method described in my fourth "Study." *Syromastes* is an exceptionally favorable form for study, and the diagrammatic clearness with which the chromosomes appear in many of the sections precludes, I think, the possibility of error in respect to the number.

---

[1] "Studies on Chromosomes, IV.," *Journ. Exp. Zool.*, VI., 1, 1909.

In my description of the male groups I emphasized the fact that the two components of the "accessory" are of slightly unequal size (as is shown in the photographs accompanying the paper), and that they are recognizable in the spermatogonial groups as two separate chromosomes which are the second and third smallest of all the chromosomes. In the female groups each of these chromosomes is represented by a corresponding pair (black in the figures). In most of the ovarian groups the smaller two are readily recognizable, and in some cases, though not always, this is also true of the larger pair. The numerical and size-relations are such as to show that after maturation the egg must contain one member of each of these pairs. Though nothing is directly known of the maturation-process in the female, it may be inferred with probability that in synapsis the two larger and the two smaller of these pairs unite to form two corresponding bivalents, which may be designated as *aa* and *bb* (*II* and *ii*,

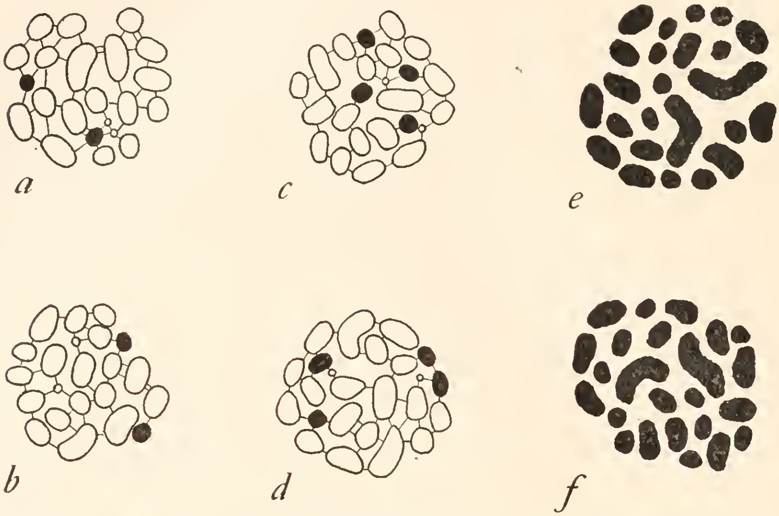


FIG. 1. *a, b, Syromastes marginatus* L., spermatogonial chromosome-groups; *c, d,* ovarian groups of the same; *e, f, Pyrrochoris apterus* L., ovarian chromosome-groups.

in the terminology of my former paper). By the subsequent disjunction of each of these pairs the mature egg receives *a* and *b* in addition to 10 other chromosomes. Fertilization of the egg by a spermatozoön containing the "accessory" $(a + b)$ will therefore give the characteristic female group $(a, b, a, b + 20)$, while fertilization by one that lacks $a + b$ will give the male group $(a, b + 20)$. For the sake of comparison two of the spermatogonial groups are reproduced in Fig. 1, *a, b*. In the figures of both sexes the chromosomes identified as *a* and *b* are made black. The essential relations in both sexes are shown in the diagram,

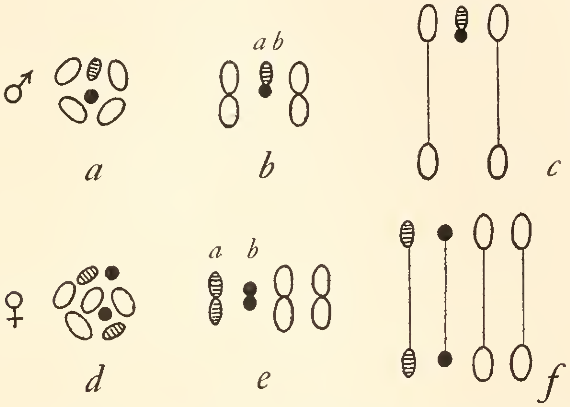


FIG. 2. Diagrams of maturation in *Syromastes*. *a*, spermatogonial chromosomes (actual number 22); *b, c*, spermatocyte-division; *d*, ovarian chromosomes (actual number 24); *e, f*, maturation division (inferred).

Fig. 2, in which *a* is cross-barred, *b* black, and the other chromosomes (of which only four are represented) are in outline.

It is evident that, except for the different total number of chromosomes in the two species, these phenomena are essentially similar to those seen in *Phylloxera fallax*, which likewise has two "accessories" and two more chromosomes in the female somatic groups (12) than in the male (10). In *Phylloxera caryæcaulis* the two "accessories" are of unequal size, as in *Syromastes*, but the phenomena are complicated by the fact that these two chromosomes are often united in the somatic groups of the male, and are apparently always thus united in those of the female. In the male they are always united at the time of the spermatocyte-divisions to form the "accessory," which is in reality double, like that of *Syromastes,* and sometimes separates into its two components as it moves to the pole. Morgan therefore concludes that the true numbers of the chromosomes in the two sexes of this species are eight and six respectively, though the female seems to show but six and the male either five or six (according as the two "accessories" are united or separate).

The point to which I would call attention is the similarity between *Phylloxera caryæcaulis* and *Syromastes* in respect to the mode of synapsis. In the spermatogenesis of both forms the two unequal "accessories" unite in synapsis (if the process can properly be so called) to form the bivalent *ab,* consisting of two unequal components, which passes into the female-producing spermatozoa. In the maturation of the male-producing egg of *Phylloxera,* however (and apparently the same must be true of the sexual eggs), a different process takes place, the two larger and the two smaller components uniting to form the bivalents *aa* and *bb,* again exactly as there is reason to conclude in the case of the egg of *Syromastes.* In *Phylloxera,* as Morgan points out, this involves a redistribution of the four chromosomes, since in the somatic groups they are united to form *ab* and *ab,* but recombine at the maturation period to form *aa* and *bb.* This remarkable redistribution, I think, loses much of its anomalous character on comparison with the facts in *Syromastes,* where *a* and *b* are always separate in the somatic groups.

These facts, together with those determined by Payne in *Fitchia* and other forms (now in press in this journal) and my own earlier ones on *Thyanta,*[1] which shows essentially the same conditions as in *Fitchia,* lead me to a somewhat different interpretation of the "accessory" chromosome in *Syromastes* from that given in my fourth "Study." In that paper I adopted the conclusion that the two components of the bivalent "accessory" were identical respectively with the large and small "idiochromosomes" of such forms as *Metapodius* or *Lygæus.* I did not then see that all the facts are equally consistent with the view that these two components, *taken together,* represent the single odd chromosome of *Anasa, Protenor* and other similar forms, and that the small idiochromosome has disappeared. Payne discovered that in the reduvioids, where a single small idiochromosome or "Y-element"[2] is always present, the large idiochromosome is in some species a single chromosome (*Diplocodus*), in others is represented by two (*Fitchia, Conorhinus*) or three chromosomes (*Prionidus*), and in the galgulid genus *Gelastocoris* (*Galgulus*) by four chromosomes, which behave in maturation as a single unit (X-element) that is obviously comparable to a single large idiochromosome in its relation to sex-production. Payne concludes, with great probability, that the double or multiple X-element in these forms has arisen by the separation of an originally single large idiochromosome (such as still exists in related species) into two or more components. In *Conorhinus,* where the X-element is double, the two components are unequal in size, and by the disappearance of the Y-element a condition would arise closely similar to that seen in *Syromastes.*

---

[1] Reported at the meeting of the American Society of Zoölogists in December, 1906, but still unpublished.

Whether such has been the actual mode or origin in *Syromastes* or not, it seems probable that here too the double "accessory" was originally a single chromosome that has separated into two parts, which still act as a unit in the maturation divisions and retain the same relation to sex-production as the original one. *Phylloxera caryæcaulis* may plausibly be regarded as in process of transition from the condition in which a single "accessory" chromosome is present (as appears to be the case in the aphids) to one in which it has separated into two parts, as in *Syromastes.*

I will add a brief account of the female groups in *Pyrrochoris apterus* L., material for which has also been obtained through Professor Boveri. A reëxamination of the male groups (Wilson, Study IV.) showed the spermatogonial number to be 23, including a single unpaired idiochromosome ("accessory" chromosome) which is at once recognizable from the fact that it is nearly twice the size of any of the other chromosomes. This passes into half the spermatozoa, which receive 12 chromosomes, while the others receive but 11, as was originally described by Henking. The female groups clearly show 24 chromosomes, of which two are of the same relative size as the unpaired one of the male. Two of the ovarian groups, typical of many others observed, are shown in Fig. 1, *e, f*. These facts show that *Pyrrochoris* conforms to the ordinary type in which the male has an odd chromosome, as in *Anasa, Protenor, Alydus, Largus* and many others.

---

[2] In a recent general discussion I have used the terms "X-element" and "Y-element" to designate respectively the large and small idiochromosomes, or their homologues, whether they consist of a single chromosome or of more than one. See *Science,* XXIX., 732, January 8, 1909.

Columbia University,
January 23, 1909.

OBSERVATIONS ON THE GERM CELLS OF HYDRA.

GEO. W. TANNREUTHER.

The germ cells of hydra, which pass through stages comparable to those of higher animals, possess some peculiarities that have not been previously mentioned. Some investigators advocate the specificity of the germ cells, while others claim that the sex cells are the immediate derivatives of the ordinary interstitial cells.

Kleinenberg (4), Hertwig (3), Brauer (1) and others consider the sex cells as interstitial in origin and state that the egg first becomes recognizable after the ovary has begun to develop. Downing (2) claims that there is continuity of germ plasm in the sense of a specific line of germ cells, that the egg (*Hydra fusca*) is always present even before the interstitial cells begin to form the ovary and that the egg may grow rapidly and take the initiative in its formation. He furthermore believes that the egg is recognizable as such in the adult hydra and in general that in some stage in the embryonic development certain cells are stamped with sex characters, so that they and their progeny form the sex cells distinct throughout the life of the individual.

A careful examination of sections from *Hydra* sp.? (Brauer) gives pretty conclusive evidence that not only the egg but the sperm as well is interstitial in origin. There can be no question in case of the sperm, as the different stages in development can readily be traced from the interstitial cells to the mature sperm. Furthermore, the progenitors of the spermatozoa have no special characters by which they can be recognized as germ cells. The cells that give rise to the eggs are interstitial in position and can be distinguished in the adult hydra from the ordinary interstitial cells by their large nucleus, nucleolus and abundance of chromatin, even before the growth of the ovary begins, as Downing states. This is especially true during the breeding season. If these sex cells could be distinguished during the budding season as well, it would at least suggest specificity of the germ cells.

In the diœcious form *Hydra* sp.? (Brauer) the egg cells cannot be distinguished from the ordinary interstitial cells except during the period of sexual reproduction. Fig. 1, *a* and *b*, represents two adjacent eggs at the stage of development when they first become recognizable. They have begun to enlarge and can readily be distinguished from the adjoining cells by their size and vacuoles next the nucleus. The above figure was taken from an adult hydra in which six eggs were present in different stages of development, ranging from the interstitial cells to the undisputed egg. These eggs are isolated or found in groups. When two or more are found adjacent the cell walls become dissolved and one persists as the developing egg (Tannreuther). In a few instances observed two of these adjacent cells persisted and gave rise to two mature eggs. These cases, however, are extremely rare in proportion to the number of eggs produced.

The above results do not warrant the view that there is continuity of germ plasm in *Hydra*. Until sex cells distinct from the somatic can be traced through successive generations, we have no positive evidence of such a continuity.

The different generations of the sex cells of *Hydra* are distinct and can readily be recognized. In the formation of the egg there is a distinct growth period. Reduction occurs at the end of the growth period just before the first polar body is formed. The polar bodies remain attached after cleavage has begun, by means of a single cytoplasmic thread. The first polar body is larger than the second.

The spermaries are composed of an indefinite number of cysts. The individual cysts originate from a single or several interstitial cells. Fig. 2 represents a longitudinal section of a single cyst containing spermatogonia, which have originated from a single interstitial cell. After the spermatogonia have divided a few times (the number of divisions varies in different individuals) those found at the distal end of the cyst become transformed into the spermatocytes of the first generation without growth. In Fig. 3 the spermatocytes of the first generation are recognized by their dense chromatin mass. Different stages of development are found in a single cyst, ranging from the spermatocytes of the first generation to mature sperm, as shown in Fig. 4. The successive zones of the developing sperm are distinct. The nuclei at the extreme proximal end of the cyst cease dividing and become the nuclei of new interstitial cells after the spermaries have disappeared.

My observations on the nuclear changes in the different stages of development confirm those of previous investigators. Reduction occurs after the last spermatogonial division just before the spermatocytes of the first generation divide. In the division of the nuclei of the spermatocytes of the first and second generations, the cell wall remains intact and produces a multinucleate cell (Figs. 5–7), and give rise to four sperm within a common

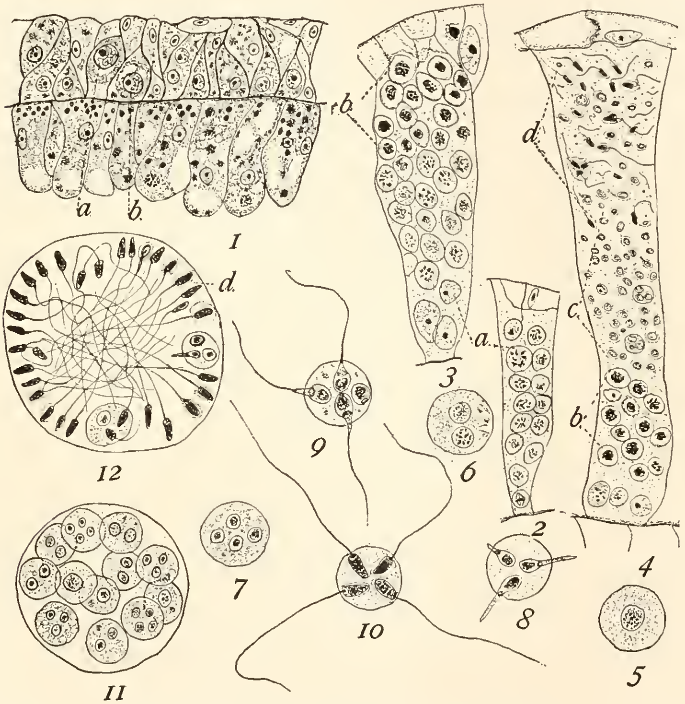


Fig. 1. Longitudinal section showing two developing eggs. *a* and *b*, eggs.

Fig. 2. Longitudinal section of a single cyst.

Fig. 3. Section of cyst little later than preceding. *a*, spermatogonia; *b*, spermatocytes of the first generation.

Fig. 4. Longitudinal section of cyst showing maximum development. *c*, spermatocytes of the first and second generations; *d*, mature sperm.

Figs. 5–7. Formation of spermatids within common vesicle.

Figs. 8–10. Developing sperm. Figs. 5–10, from living cells.

Figs. 11 and 12. Cysts removed from living spermary.

vesicle. The vesicular wall is very thin and the four sperm pass through it when mature and become free within the distal end of the cyst (Fig. 4). The mature sperm are very active within the cyst and finally escape to the exterior through a small temporary opening of the spermary. After the mature sperm have all escaped, the opening closes until more sperm mature. When the spermaries are extremely large, this process continues from forty to fifty hours.

In order to show the four sperm within a common vesicle, it is necessary to dissect the living spermary apart, as the sperm always escape from the common vesicle before passing to the exterior. The individual cysts when separated become more spherical (Figs. 11 and 12), and the different stage of development in the living sperm can easily be distinguished.

Kleinenberg in his description of the sperm in *Hydra viridis* definitely states that the sperm are formed from interstitial cells that have divided a number of times; ultimately the nucleus of the cell (the spermatocyte of the first order) disintegrates while the cell substance becomes granular, and in place of the nucleus there appears from one to four refractive bolies, which give rise to the sperm. The refractive bodies referred to beyond doubt result from the two divisions of the nucleus, which he thought disintegrated. The four nuclei (spermatids) within the common vesicle do have the appearance of refractive bodies, especially in the living material.

Korotneff (5) gave similar results. He states that the sperm form directly from the nuclei of a multinucleate mother cell. Downing (2) does not mention this interesting phenomenon, which is found in *Hydra viridis* and *Hydra* sp.? (Brauer).

The mature sperm possess extreme vitality and may remain active from one to three days after escaping from the spermary. The mature sperm of any individual spermary possess about the same degree of fertility. No degenerating spermatogonia were found.

In the monœcious form *Hydra viridis* the spermaries become mature before the ovaries. Occasionally sperm and ova on one individual would ripen at the same time, making self-fertilization possible. In order to prove that self-fertilization did occur, individuals with both spermaries and ovaries at the same stage of development were isolated and placed in distilled water for one hour, in order to kill any mature sperm that might adhere from other individuals. The *Hydra* were then placed separately in water free from sperm. The spermaries and ovaries matured and self-fertilization took place. The cleavage was normal.

Summary.

1. *Hydra* sp.? (Brauer) does not show continuity of the germ plasm.

2. The eggs can be distinguished from the interstitial cells in the adult *Hydra* before the ovary is formed. This is especially true during the breeding season.

3. The different generations in the formation of the germ cells are comparable to those of higher animals.

4. In the division of the spermatocytes of the first and second generations the cell wall remains intact and the four spermatids are formed within a common vesicle, each producing a mature sperm.

5. Self-fertilization occurs in *Hydra viridis*.

Zoölogical Laboratory,
University of Missouri.

BIBLIOGRAPHY.

1. **Brauer, A.**
'91 Ueber die Entwicklung von Hydra In. Z. f. Wiss., Zool., 52, 2.
2. **Downing, E. R.**
'04 The Spermatogenesis of Hydra. Zool. Jahr., Band 21.
'08 The Ovogenesis of Hydra fusca—A preliminary paper. Biol. Bulletin, Vol. XV., No. 2.
3. **Hertwig, R.**
'06 Ueber Knospung und Geschlechtentwicklung von Hydra fusca. Biol. Centralbl., Vol. 26.
4. **Kleinenberg, N.**
'72 Eine anatomisch-entwicklungsgeschichtliche, Untersuchung. Leipzig.
5. **Korotneff, A.**
'83 Zur Kentniss der Embryologie der Hydra. Z. Wis. Zool., Bd. 38.
6. **Tannreuther, Geo. W.**
'08 The Development of Hydra. Biol. Bulletin, Vol. XIV., No. 5.

BUDDING IN HYDRA.

GEO. W. TANNREUTHER.

Budding in hydra has received the attention of only a few investigators. Our principal information as to the origin of the bud is derived from Lang's[1] account. He describes the bud as beginning by an increase in volume and division of the interstitial cells. After the ectoderm becomes thickened, the mesoglea disappears and the cells pass from the ectoderm into the endoderm. This process continues until the ectoderm becomes reduced to its normal thickness. The mesoglea then reforms and a cavity appears in the thickened endoderm, which becomes the enteron of the new individual.

The main object of the present paper will be to give a brief account of the origin and development of the buds in hydra, more especially their manner of growth and what cells contribute most to their rapid formation.

The species studied, *Hydra viridis* and *Hydra* sp.? (Brauer) differ somewhat from Lang's account. The mesoglea does not disappear and the ectodermal cells do not pass into the endoderm. The bud, however, begins by an increase in volume and division of the interstitial cells. After they have increased once or twice in volume, as shown in Fig. 1, there is a slight outbulging of the ectoderm, which is scarcely perceptible. Fig. 2 represents the condition of the interstitial, ectodermal and endodermal cells in the origin of the bud as they appear more highly magnified. A few mitotic figures are visible. No amitotic divisions were observed. The endodermal cells contain numerous food particles, which may pass intact through the mesoglea into the ectoderm. The cells directly concerned in the formation of the bud are well supplied with food, while the remaining cells of the parent hydra show a scarcity. Many of the endodermal cells in the distal half of the hydra have a glandular appearance and are most active in the secretion of the digestive fluid, which aids in the breaking up of the food in the enteron, while those endodermal cells in the region of growth, "the formation of buds and sexual organs," are the most active in ingesting the partly digested food from the enteron and preparing it for diffusion into the ectodermal cells.

---

[1] Lang, Albert, "Uber die Knospung bei *Hydra* und einigen Hydropolypen," *Z. wiss. Zool.*, Vol. 54, 1892.

The ectoderm and endoderm in the early formation of the bud, which are quite uniform, soon become differentiated into two dis-

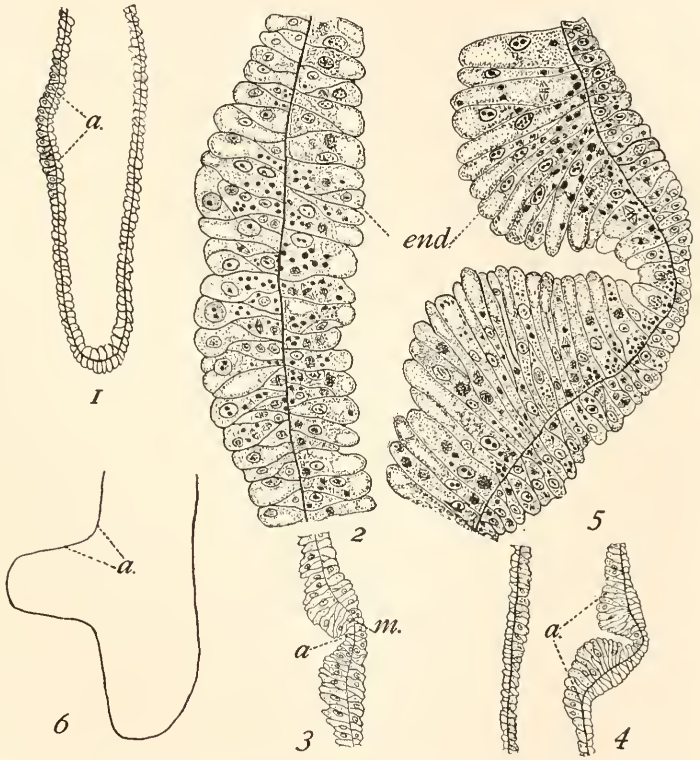


Fig. 1. Longitudinal section of hydra showing the origin of bud at *a*.

Fig. 2. Part of preceding figure at *a*. × 80.

Fig. 3. Longitudinal section through wall of hydra showing curvature of mesoglea and beginning of enteron in forming bud. *m*, mesoglea; *a*, enteron.

Fig. 4. Stage of development a little later than preceding. *a*, the point of junction between parent and bud.

Fig. 5. A portion of preceding figure at *a*, which includes the entire area in the formation of bud. *end.*, endoderm. × 90.

Fig. 6. Diagram of a longitudinal section of parent hydra with bud. *a*, active growing region in formation of bud.

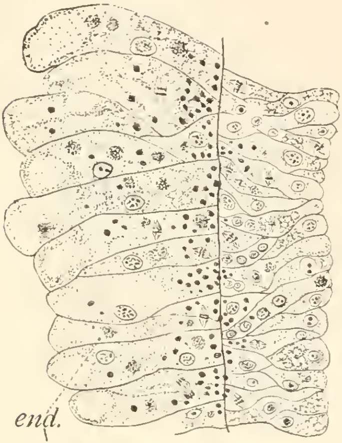
tinct regions. The cells more centrally placed (Fig. 3, *a*), which correspond to the distal end of bud, become inactive, while those on either side of the central region continue active, divide rapidly and contribute almost entirely to the rapid growth of the bud. There is a slight curvature in the mesoglea (Fig. 3, *m*), and the enteron of the new individual becomes apparent. In a stage little later than the preceding (Fig. 4), the relation of bud to parent and the condition of cells is more plainly represented. Fig. 5, a portion of Fig. 4 at *a*, represents the entire area that contributes directly to the growth of the bud. The cells at the apex of the forming bud are small. Those on either side are larger and more active in the process of division and growth. Their contents is very similar, showing an abundance of food material and cytoplasmic granules.

The ectoderm and endoderm at the junction of the parent and bud (Fig. 7) divide very rapidly and become the most active growing region in the production of the new individual. The remaining cells of the bud seldom divide. The formation of the tentacles is similar to that of the buds. The cells corresponding to their basal ends take the most active part in their growth. The mouth is formed at the distal end of the bud by a breaking through of the ectoderm.

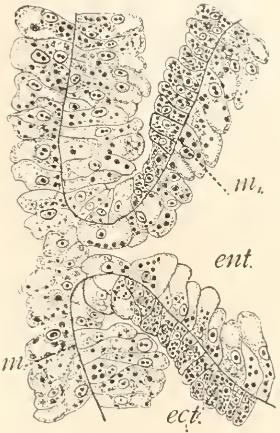
The rate of growth of the bud is determined by the amount of food present. In an active feeding hydra, the buds are often completely formed in thirty to forty hours. While in those hydras with a moderate supply of food the buds grow very slowly and may require four or five weeks or even more time for their complete development. In the latter instance after the buds are nearly formed, they will be absorbed in the absence of food. In the process of absorption the cell walls of the bud become imperceptible and the cell contents presents the appearance of a complete syncytium. When the buds are nearly absorbed, if the hydra is again supplied with food, the buds very seldom reform. In a few instances the buds were neither absorbed nor reformed, but remained attached to the parent as permanent individuals. Tentacles were formed later.

When the buds have reached their complete development the ectodermal cells at the proximal end undergo a rapid change

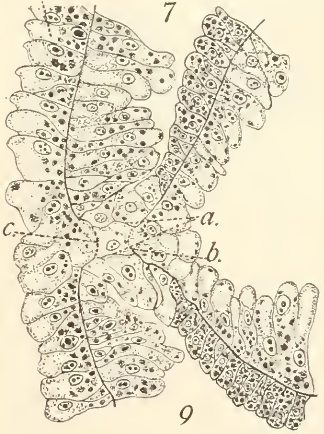
(Fig. 8, *ect*). They become more narrow, elongated and present the appearance of glandular cells. They have the power to



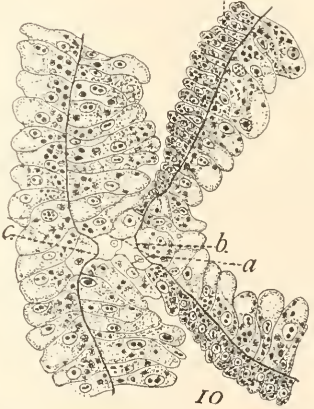
7



8



9



10

FIG. 7. Longitudinal section at junction of parent hydra and bud, taken at *a* in Fig. 6. *end.*, endoderm. × 100.

FIG. 8. Longitudinal section through base of mature bud and wall of parent hydra. m_1, mesoglea of bud; *m*, mesoglea of parent; *ect.*, glandular ectoderm; *ent.*, enteron of bud.

FIG. 9. Longitudinal section showing change of mesoglea in separation of parent and bud at *a*, and formation of new mesoglea at *b* and *c*. The endodermal cells at proximal end of bud have united. *a*, mesoglea between bud and parent becoming thinner; *b*, new mesoglea of bud; *c*, new mesoglea of parent.

FIG. 10. Longitudinal section showing completion of mesoglea at *b* and *c* and persistence of old mesoglea at *a*.

secrete a sticky substance before the bud becomes separated from the parent.

The first step in the process of separation of bud from parent occurs in the mesoglea. Fig. 8 represents the condition of mesoglea and cells before separation begins. The mesoglea, which connects the bud with parent, is uniform throughout. But almost immediately it becomes thinner and thinner until it is indistinguishable from the ordinary cell wall (Fig. 9, *a*). The enteron leading from the parent to the bud becomes discontinuous by the union of the endodermal cells. New mesoglea is now formed at the extreme basal end of the bud (Fig. 9, *b*) and at the point of former union with the parent at *c*. The mesoglea, which is more of a gelatinous nature, increases in thickness by means of secretion from the endodermal cells and soon reaches its normal condition (Fig. 10, *b* and *c*). After the formation of the mesoglea is complete, the bud remains attached to the parent by a few ectodermal and endodermal cells, as shown in Fig. 10. The former connecting mesoglea is represented by the dotted lines at *a*. The cells between the dotted lines, which are endodermal, become external to the newly formed mesoglea and take the position of ectoderm. Whether these cells persist or not and function as ectoderm is difficult to say, as there is no possible means of following them in the process of separation of bud from parent.

SUMMARY.

1. The initial step in the formation of bud in hydra is found in the interstitial cells.

2. The most active region of growth in the formation of the bud is found at the junction of the forming bud and parent, where the cells divide very rapidly and contribute almost entirely to its growth.

3. When the bud is nearly formed the ectodermal cells in the basal region become transformed into granular glandular cells, which later secrete a glutinous substance for attachment of hydra.

4. The rate of growth in buds is controlled by the amount of food present. Starvation after buds are nearly complete often causes their complete absorption by parent hydra.

Zoölogical Laboratory,
University of Missouri.

Vol. XVI. April, 1909. No. 5.

BIOLOGICAL BULLETIN

THE NUCLEOLI IN THE SPERMATOCYTES AND GERMINAL VESICLES OF EUSCHISTUS VARIOLARIUS.

KATHARINE FOOT AND E. C. STROBELL.

I. In the resting first spermatocyte of many insects there appears a spherical, chromatic body which invariably stains as intensely as the chromosomes.

McClung ('02) was the first investigator to draw special attention to this structure by his suggestion that it may function as a sex determinant.

A majority of the cytologists who have studied this structure interpret it as one of the chromosomes which persists through the rest stage of the first spermatocyte, and they claim that its presence or absence in a spermatozoön is the determining factor of sex, McClung assuming that its presence in a spermatozoön causes the fertilized egg to produce a male, whereas Stevens, Wilson and others affirm that its presence in a spermatozoön causes the fertilized egg to produce a female, and in those cases where two (a large and a small) chromatin nucleoli are present, the larger is the female-determining, and the smaller, the male-determining factor.

Identifying the chromatin nucleolus as a chromosome enlarges at once the sphere of the problem, involving this structure in the maze of hypotheses and theories associated with the chromosomes. This identification of the chromatin nucleolus as a chromosome is of great importance, because it presumably demonstrates that a definite chromosome retains its individuality throughout the rest stage, and it presents very strong evidence for the theory of the individuality and continuity of the chromosomes — the theory which is the corner-stone of all hypotheses involving the claim that the chromosomes are the cause rather than the expression of cell activities.

We believe that the evidence we find in *Euschistus*, as well as in *Anasa* ('07), is distinctly opposed to the interpretation that the chromatin nucleolus of the resting first spermatocyte is a phase of one or more of the chromosomes, but we shall reserve the publication of this evidence in *Euschistus* until we can control it by a more complete comparison with other forms. In the present paper we shall limit ourselves to the relatively simple question, is the chromatin nucleolus a structure associated with the male cell only, as claimed by McClung, or is it, as claimed by Wilson, a chromosome received from the egg, which during the rest stage appears in the form of a chromatin nucleolus?

Two chromatin nucleoli unequal in size have been demonstrated by Montgomery ('98, '01, '06) in the first spermatocytes of several varieties of *Euschistus*, including *Euschistus variolarius*, and Wilson ('05) has demonstrated these structures in other Hemiptera, naming them chromosome nucleoli, because he believes them to be chromosomes. In describing them in *Cœnus* and *Lygæus* he says: "Throughout the whole of the growth period in *Cœnus*, and from stage *e* onward in *Lygæus*, at least one of the idiochromosomes can always be distinguished as a compact, spheroidal, intensely staining chromosome nucleolus and frequently both idiochromosomes are distinguishable in this form in all of these stages. . . . It is clear beyond all question that at least the large idiochromosome may retain its identity throughout the whole growth period, with the small idiochromosome the case is not so strong" (p. 389). Of *Brochymena* he says: "There can be no doubt that when only one chromosome nucleolus is present it is to be considered as a bivalent body arising by the fusion or synapsis of the two idiochromosomes" (p. 392).

In the resting first spermatocyte of *Euschistus variolarius* the chromatin nucleolus is the most deeply stained and conspicuous structure in the cell (Photos 1–10). As a rule, only one is present, but quite frequently there are two, sometimes equal, but in most cases, very unequal in size; exceptionally we have found three, or even four, nucleoli in the same nucleus.

We roughly estimated the number of the nuclei with one or more chromatin nucleoli, by counting 625 cells in one testis, and of these cells 591 showed one nucleolus, 27 two nucleoli, and 7 three nucleoli. These 625 cells represented only a small area of the preparation and we did not repeat the experiment on another testis — the estimate, therefore, offers merely an indication that in a large majority of cases only one chromatin nucleolus is present.

By a comparative study of the spermatocytes and oöcytes of the same form we ought to find an answer to the question, whether the chromatin nucleolus is associated with the male cell only, or whether it has its origin in the oöcyte.

As far as we are aware, the germinal vesicles shown in Plates I., II. and III. are the first that have been demonstrated in any of the Hemiptera heteroptera. A great deal has been written about the spermotocytes and important generalizations have been drawn from the behavior of the chromatin nucleolus in these male cells, but not one word has been said about the corresponding, very important stages in the female, although we have been confidently assured that the chromatin nucleolus in the male has been derived from the egg. If this is true, it would seem at least logical to expect to find some evidence of its presence in the egg at the stage of development corresponding to the stage in which it is so conspicuous in the male cell.

Even if we assume for the sake of argument that the chromatin nucleolus is a chromosome, we still do not avoid the logical expectation of finding it in the oöcytes, for if one of the chromosomes of the spermatocyte possesses the distinguishing trait of persisting through the rest stage in the form of a chromosome nucleolus, and if that chromosome is a chromosome contributed to the male cells from the mother, we should expect to see its distinguishing characteristic manifested in even a more marked degree in the oöcyte. And further, as the chromosome nucleolus of the spermatocyte is claimed to be only one of a pair of chromosomes — its mate being contributed by the egg at the time of fertilization — we should expect to find in the resting oöcyte a pair of chromosomes represented by two univalent, or one bivalent chromosome nucleolus.

In the case of *Euschistus* we are told that the larger of the two chromatin nucleoli of the spermatocyte is the homologue of the accessory chromosome of other forms, and if this interpretation is correct we may expect to find a large bivalent or two univalent chromatin nucleoli in the growing oöcytes.

II. We find the chromatin nucleolus of the spermatocyte persisting through the entire growth period, Photos 1 to 10. All the resting spermatocytes shown in these photos, are from testes which contain many first spermatocyte metaphases, in which the idiochromosomes show the typical inequality in size. We have selected preparations of the resting spermatocytes showing variations in the relative size of the two chromatin nucleoli (when present) in order to demonstrate their frequent lack of conformity to the size relations of the idiochromosomes. Photo 10 shows a nucleolus persisting until the chromosomes are formed and we have several photographs demonstrating its presence to a still later stage, where the seven bivalents can be counted, but we shall reserve the discussion of these later stages for a subsequent paper.[1]

As the chromatin nucleoli of the first spermatocyte of *Euschistus* persist through the entire growth period, we certainly have a right to expect to find in the egg the equivalent of the large chromatin nucleolus during these same stages, if as asserted it owes its origin to the egg. Again, if the so-called male sex-determinant (the small chromatin nucleolus) persists through the growth stages in the male cell, there seems to be no clear reason why the so-called female sex-determinant (the larger chromatin nucleolus) should not persist through the growth period of the oöcyte.

A study of the oöcytes shown in Photos 11 to 30 demonstrates that in none of these cells can be found a structure resembling in any way the chromatin nucleolus shown in the spermatocytes of Photos 1 to 10. This is clearly demonstrated in the spermatocytes and oöcytes of Plate I. There is no structure in the oöcytes of Photos 11 to 19 which resembles in the least the chromatin nucleolus of the spermatocytes of Photos 1 to 10. The only nucleolus in the oöcytes is a relatively large achromatic structure, first clearly demonstrated in the older oöcytes. It is clearly seen in the germinal vesicles of Photos 18 and 19, Plate I., and in the germinal vesicles of Photos 20 to 25, Plate II., and in Photos 26 to 30, Plate III. All these preparations show, that as a rule its position is peripheral and this is also true of the principal nucleolus of the germinal vesicles of *Allolobophora* (Photo 31). The absence of a chromatin nucleolus is conspicuous not only in the germinal vesicles of *Euschistus* but in the young oöcytes as well. Compare the young oöcytes of Photos 11 and 12 with the young spermatocytes of Photos 1 to 5.

---

[1] We have a number of photographs showing the transition stages between Photos 9 and 10, but lack of space prevents their reproduction on these plates.

In none of the oöcytes, from the earliest to the latest stages do we find a chromatin nucleolus, and its absence in the germinal vesicles of *Euschistus* is made more conspicuous by the fact that a chromatin nucleolus is invariably present in the germinal vesicles of *Allolobophora*. If we could find such a structure in the germinal vesicles of *Euschistus*, in addition to the achromatic nucleolus, the advocates of the sex determination hypothesis could justly claim it as convincing evidence in support of their theory of its female origin, even if a demonstration of its relation to one of the chromosomes was lacking.

The absence of a chromatic nucleolus in these germinal vesicles cannot be due to the technique since in this case the more delicate achromatic nucleolus would show some disturbance. This point is exemplified in the germinal vesicle of *Allolobophora* (Photo 31), for in this preparation the pricking has disturbed the large, less chromatic nucleolus, while the small, denser, chromatic nucleolus remains perfectly intact, and this condition holds true for the hundreds of germinal vesicles of *Allolobophora* we have preserved.

It therefore seems fair to assume that the technique is not responsible for the absence of a chromatin nucleolus in the germinal vesicles of *Euschistus*.

In an earlier paper[1] we ventured to call attention to the likeness of the chromatin nucleolus in the egg of *Allolobophora* to the chromatin nucleolus of the first spermatocytes of insects. Compare the chromatin nucleolus in *Euschistus*, Photos 1 to 10, with the chromatin nucleolus in the egg of *Allolobophora*, Photos 31 and 32. In both these forms the chromatin nucleolus stains as intensely as the chromosomes, and in both forms we sometimes find one and sometimes two chromatin nucleoli.[1] The comparison may be carried even a step further. We may compare the chromatin nucleolus of *Allolobophora* to the chromatin nucleolus of some forms where it is figured at one pole of the first spindle as an undivided chromosome. The chromatin nucleolus of *Allolobophora* often persists through the first division, and in such cases it is either free in the cytoplasm, near the spindle, or at one pole of the spindle in which position it strongly resembles the undivided chromosome figured in so many forms. Photo 32 shows a section through the first maturation spindle of an egg of *Allolobophora*, and a comparison of the chromatin nucleolus at the upper pole of this spindle, with the chromatin nucleolus in the germinal vesicle of Photo 31 leaves no doubt of the identity of the two structures. The identification is further confirmed by the clear demonstration in this and the adjoining sections of all eleven chromosomes in the equatorial plate of the spindle. If the chromosomes of *Allolobophora* were spherical as they appear in sections of so many forms, we could not so easily identify the chromatin nucleolus, for it stains exactly like the chromosomes. Such a case is described by Arnold ('08) in the spermatogenesis of the Coleopteran *Hydrophilus piceus*. During the rest stage the chromatin nucleolus of this form is easily recognized, but he finds it impossible to differentiate it from the chromosomes during the first prophase, because the chromosomes at this stage are also spherical and both structures stain alike. He is able to identify it again at the first metaphase, where it lies in the cytoplasm near the spindle, or within the spindle at one pole. It persists through the first division, but disappears before the second mitosis.[2] It is clear that in its origin, behavior and time of disappearance the chromatin nucleolus of the spermatocytes of *Hydrophilus* closely resembles the chromatin nucleolus of *Allolobophora*, with the exception that the latter does not invariably persist through the first division. Arnold ('08) supports Moore and Robinson's ('05) conclusion that the chromatin nucleolus of the first spermatocyte is not a chromosome and does not take part in either division. His evidence for this is very convincing, he says, if it is to be interpreted as an accessory chromosome which remains undivided in the first spindle it should divide in the second spindle, and half the second spindles should show one more chromosome than the other half. Arnold finds, on the contrary that the number of chromosomes in the second spindle is as constant as the number in the first. He gives in the following table the results he obtained:

---

[1] Foot and Strobell ('05).

---

[1] Foot and Strobell ('05), Photo 115.

[2] Wheeler ('97) figured the nucleolus of the germinal vesicle of *Mysostoma glabrum* persisting through the second cleavage and he says that in some cases it persists through the next stage, and perhaps even later, page 35.

Out of 100 counts of first meiotic:

85 per cent. gave....... 15 gemini and the nucleolus.
7 “ “ “ over 15 “ “ “ “
8 “ “ “ under 15 “ “ “ “

Out of another 100 counts of first meiotic:

90 per cent. gave....... 15 gemini and the nucleolus.
3 “ “ “ over 15 “ “ “ “
7 “ “ “ under 15 “ “ “ “

Out of 100 counts of second meiotic:

91 per cent. gave................. 15 chromosomes.
6 “ “ “ over 15 “
3 “ “ “ under 15 “

The resemblance between the chromatin nucleolus of the first spermatocyte of insects and the chromatin nucleolus in the germinal vesicles of some forms has been noted by Häcker ('07) who says, that in those cases in which the heterochromosome is figured as a cap-shaped mass on a large pale plasmosome, the two structures are extraordinarily like the two kinds of nucleoli in the germinal vesicles of the lamellibranch type. He says: “So kann man sich dem Eindruck nicht entziehen, das z. B. das, ‘akzessorische Chromosom’ in den ruhenden Spermatocyten von Scolopendra (Blackman, 1905, tab. 2, fig. 10–15) früher einfach als ‘Nucleolus’ beschrieben worden wäre, und man ist geneigt, sich zu fragen, ob denn in allen vorliegenden Angaben bereits eine reinliche Scheidung zwischen den Heterochromosomen und den echten Nucleolen (Plasmosomen) durchgeführt ist. Es sei mir auch gestattet, darauf hinzuweisen, das die von Wilson u. a. gegebenen Bilder, in welchen ein Heterochromosom als kappenförmige Masse einem grossen Plasmosom aufgelagert erscheint, eine auserordentliche Ähnlichkeit mit den Befunden in den Keimbläschen des Lamellibranchiaten-Typus zeigen, d. h. in denjenigen Keimblässen, in welchen sich zweierlei Nucleolen von verschiedener Lichtbrechung, Quellbarkeit und Tingierbarkeit befinden."

It is a suggestive fact that in *Allolobophora* we have an hermaphrodite form, and if one is determined to regard the chromatin nucleolus of the spermatocytes as a sex-determinant, some interesting conclusions might be drawn from a comparison of the two types of nucleoli observed in this hermaphrodite form, with the two types characteristic of the male and female cells in *Euschistus*. Compare for example the large nucleolus in the germinal vesicle of *Allolobophora* (Photo 31) with the large achromatic nucleolus of the germinal vesicles of *Euschistus* (Photos 18 to 30), and compare further the smaller chromatin nucleolus of *Allolobophora* (Photos 31 and 32) with the chromatin nucleolus of the spermatocytes of *Euschistus* (Photos 1 to 10).

Even if we could be sure that in *Euschistus* the achromatic nucleolus of the egg cells is not represented in the male cells, and that a striking likeness exists between the male and female nucleoli of *Euschistus* and the two types of nucleoli in the germinal vesicles of *Allolobophora*, we would still be unable to claim from the comparison any results of general significance, for the reason that the observations of many investigators of the spermatogenesis of insects offer contradictions to an assumption that the achromatic nucleolus is associated solely with the female cell — these observers having figured a pale, achromatic nucleolus in the spermatocytes, in addition to the chromatic nucleus characteristic of these cells. As opposed to these facts, however, other investigators have found no structure in the spermatocytes that can be interpreted as a nucleolus, other than the characteristic chromatin nucleolus.[1] These conflicting observations, if equally reliable, compel the conclusion that individual forms may differ as to the absence or presence of an achromatic nucleolus.

---

[1] In his "Studies on Chromosomes — IV.," *Jour. Exp. Zoöl.*, Vol. VI., No. 1, 1909, Wilson in describing *Syromastes*, does not mention a large pale plasmosome, such as he has described in other forms. In *Pyrrochoris* he states that there are from one to three nucleolar-like bodies, which on account of the staining reactions, he believes to be plasmosomes, page 82.

In *Euschistus* we have not been able to demonstrate its presence at any stage of the growth-period of the spermatocytes. In sections we often find faintly staining areas that might be interpreted as an achromatic nucleolus, but in view of the possibility of artefacts in such preparations, we hesitate to interpret them as true nucleoli, unless we can support the interpretation in our smear preparations. Until this point can be settled we are not justified in drawing any conclusions from the obvious difference in type between the nucleoli in the male and female cells of *Euschistus*, though we have here quite as marked a sexual difference of the nucleoli, as any that has been shown for the chromosomes, a difference that appears in no way associated with the maturation divisions of either germ cell.

In comparing the achromatic nucleolus of the oöcytes of *Euschistus* with the principal nucleolus of *Allolobophora* we find some individual differences between the two. The principal nucleolus of *Allolobophora* is clearly differentiated in the young oöcytes as a small, dense chromatic body, and it can be traced uninterruptedly through the entire growth period of the oöcytes. During this period it increases in size, proportionately to the growth of the nucleus, gradually becomes less dense and less chromatic, and finally, when the germinal vesicle has reached its maximum size the principal nucleolus often appears as in Photo 31. In some cases, however, its dense and chromatic character, distinctive of the earlier stages, persists until the chromosomes are fully formed or again it may so completely disintegrate that only a clear space remains as evidence of its existence.[1]

The achromatic nucleolus of *Euschistus* differs from the principal nucleolus of *Allolobophora* in not being present in the young oöcytes as a small dense chromatic nucleolus. We have been unable to demonstrate any such chromatic body in the young oöcytes of *Euschistus*. In the youngest stages in which a nucleolus is found, it appears as clearly achromatic as when it has reached its maximum growth in the germinal vesicle — compare Photos 13 and 16 with Photos 18 to 30. There are also indications that it is often not formed until after the oöcytes have attained a definite growth, for in such clear preparations as are shown in

[1] Foot and Strobell ('05), Photo 122.

Photos 11, 12, 14, 15 and 17, there is no evidence that such a structure is present.[1]

If the nucleolus in the germinal vesicle of *Euschistus* corresponds to the principal nucleolus of *Allolobophora* it must be conceded that there are marked individual differences between the two, differences quite as marked as those existing between the chromosomes of these two forms.

INDIVIDUALITY AND CONTINUITY OF THE CHROMOSOMES.

The investigators who question the individuality and continuity of the chromosomes urge the necessity for further study of the critical stages bearing on this problem — the stages occurring between the end of one mitosis and the beginning of the next. Meves ('08) holds that during these stages it is impossible to recognize the chromosomes. He says: "Man sucht hier, wie O. Hertwig 1890 geschrieben hat, in den Kern etwas hineinzudemonstrieren, was kein unbefangener Beobachter in seiner Struktur erkennen wird.

Fick ('07) claims, that a study of the rest stages proves that the theory of the individuality and continuity of the chromosomes is untenable.

Tellyesniczky ('07) also makes a strong plea for more thorough and careful work on these stages. We are in sympathy with him in his skepticism concerning the individuality and continuity of the chromosomes, but our results differ from his in some, perhaps unimportant, details — differences which may normally exist in different forms. For example Tellyesniczky ('05) believes that a homogeneous distribution of the nuclear substance throughout the nuclear vesicle precedes every mitosis, and the young spermatocytes of our Photos 1, 4 and 5 lend support to this claim. The young oöcyte of Photo 13 also indicates a diffused condition of the chromatin, but as a rule the chromatin of the young germinal vesicles appears as fine granules often very evenly distributed throughout the nucleus as in Photos 11, 14 and 17. Possibly this granular condition may be an artefact — one phase of a precipitate left after drying, for in some cases the chromatin of the young oöcytes is as homogeneous as that of the spermatocytes of Photos 1, 4 and 5.

---

[1] Among recent observations on this point, Deton ('08) finds in *Thysanozoön Brochii* that the nucleolus is absent in the young oöcyte, first appearing at the beginning of the growth period.

We find nothing in these germinal vesicles answering to Tellyesniczky's karysomes, unless he would homologize the aggregations of chromatin shown in Photos 12, 15 and 16 with these structures. These aggregations of chromatin are too numerous to be interpreted as the seven bivalent chromosomes of the later stages, or as their fourteen component univalents. In the germinal vesicle of Photo 15, for example, there are about fifty clumps of chromatic substance. Perhaps these clumps of chromatin correspond more closely to the segregated granules Wassilieff ('07) describes in the nucleus of *Blatta*. These clumps, he says, are too numerous to be interpreted as representing individual chromosomes. Later he finds that they disintegrate and are scattered throughout the nucleus like a fine dust; the delicate threads of the later stages being formed at the expense of this dust-like substance.

In the germinal vesicles of *Euschistus variolarius* the chromosomes first appear as extremely fine threads, which seem to arise as a concentration of the distributed granular mass, these granules gradually disappearing as the threads become more defined. Photo 17 shows a germinal vesicle at the stage just before the chromosomes appear — the chromatin is still distributed as granules throughout the nucleus, although the size of this nucleus is nearly equal to many of the germinal vesicles in which the chromatic threads are fully developed. In Photos 18 and 20 we see the delicate chromatic threads just appearing, as yet very indistinct, and in the later stage shown in Photo 21 the threads are more distinct but too much tangled to warrant any precise interpretation. In later stages, however, we can often trace among the filaments, an unbroken thread, so long that it cannot possibly be interpreted as one of the fourteen univalents, it must represent at least two univalents attached end to end. Again some of these threads are long enough to justify the inference that they represent even more than a bivalent, indicating that in *Euschistus* the chromosomes emerge from the rest stage not as univalents, but as long threads representing at least one or more bivalents, thus supporting the observations of those who believe that bivalents arise by the omission of a transverse division of a spireme, rather than by a conjugation of univalents. We believe that the evidence in *Euschistus* is opposed to the interpretation that the chromosomes first appear as univalents and later conjugate, either longitudinally or end to end. We believe, rather, that there is a tendency to form a continuous spireme, because we find cases in which very long, thin, unbroken threads are present, and a few examples of this kind outweigh as evidence, a large number of cases where we find the threads broken into small pieces. These delicate threads could easily be broken by the technique, for after pricking, the germinal vesicles flatten and dry on the slide, and this disturbance, combined with the shrinkage of drying, could easily account for the rarity of the cases in which the long thin threads remain unbroken.

In Photo 24 we see this tendency to the formation of a spireme — a few of the threads can be traced certainly too far to justify any assumption that they are univalent or even bivalent chromosomes. A long unbroken thread is also demonstrated in Photos 23, 28 and 29.

Tellyesniczky ('07) claims that the long thin chromatic threads do not become shorter and thicker by contraction. He thinks rather this is accomplished by a flowing together and change of place of the substance of which the thread is composed. He says, there is a continual disintegration and rebuilding of the threads and the new structure is formed at the expense of the old.

We believe this is not the case in *Euschistus*. For example, it seems reasonable to interpret the chromosomes of Photo 30 as due to contraction of long thin bivalents (as shown in Photo 25), rather than to regard them as entirely new formations. Such a detail as this is interesting, but it seems to us only incidental to the all-important question, do the chromosomes retain their individuality throughout the rest stages — do they differ in this particular from other structures of the cell — the centrosome — the aster — the spindle — the nucleolus, etc. These elements return at each cell generation with as much regularity as to size and form as do the chromosomes, but those who believe in the individuality and continuity of the chromosomes attribute to the latter structures a causal significance not now ascribed to any other organ of the cell.

In the past, however, similar claims have been made for the centrosome — its individuality and continuity were asserted by many cytologists, some going so far as to believe the centrosome to be the cause rather than the expression of cell activity.

Certain facts indicate that all the chromatic substance of the germinal vesicle of *Euschistus* is not used for the chromosomes. We find in many germinal vesicles various segregations of chromatic substances which are present after the chromosome threads are formed and which often do not entirely disappear until the prophase stage. We sometimes find irregular granular or homogeneous masses as in Photos 20 and 22, sometimes numerous large granules, so dense that they look like very small nucleoli (Photos 19, 21, 23, 26, 27 and 29), and sometimes merely deeply staining areas, as shown in Photos 23, 24 and 27. We have no means of proving that this chromatic substance, not used for the chromosomes, is chromatin, but for many forms it has been claimed that only a small part of the chromatin is used in the formation of the chromosomes and we are therefore perhaps justified in surmising that the chromatic substance in *Euschistus* (which often persists until after the chromosomes are formed) may be chromatin residue, which assumes various forms during its disintegration and disappearance.

The early stages of the development of the germinal vesicles shown in Photos 11 to 17 we interpret as indicating that the chromosomes completely disintegrate — that any claim of their morphological persistence during these stages must be made as a pure assumption, as there is no morphological evidence whatever of their persistence. On the contrary there is every indication that they completely disintegrate. We believe we have no reason to assume that the reappearance of the chromosomes at certain stages differs essentially from the reappearance of the centrosome, aster, spindle, nucleolus and other cell structures.

Fick ('07) in his critical review of the inconclusive evidence that has been offered as proof of the individuality and continuity of the chromosomes, and of their causal character, sounds a welcome note of protest against recent chromosome speculations. In addition to Fick such experienced cytologists as Häcker and Meves, have expressed their skepticism of the sex-determination hypothesis in no uncertain terms, and many of the investigators who have studied the accessory chromosomes in insects are equally incredulous, Montgomery, Schäfer, Moore, Robinson, Arnold and others.

We have reserved a description of the later stage of the germinal vesicle chromosomes of *Euschistus* for a subsequent publication, which will discuss only the chromosome groups in both male and female cells. In the male cells we have traced the idiochromosomes through both maturation divisions and are able to support, in the main, Wilson's results as to their division in these stages. He says, the idiochromosomes remain univalent in the first division, each dividing independently, and in the second division the two separate — the small idiochromosome going to one pole and the large idiochromosome to the opposite pole. He gives no details however as to the method of the first division and as we find an interesting irregularity in this division, we believe it is worthy of consideration. As the idiochromosomes separate in the second spindle, this is presumably the so-called reduction division for this unequal tetrad, and the first division should be therefore an equation division, and *both* idiochromosomes should divide longitudinally, if the significance that has been attributed to the longitudinal and transverse divisions of the chromosomes holds true in this form.

The value of the significance of a longitudinal or transverse division is based on the assumption that the so-called ids are arranged in a row, and a morphological demonstration of this assumption is claimed for those cases in which the chromosomes are rod- or thread-shaped — these rods or threads often appearing as a single row of chromomeres.

In our smear preparations of the testes of *Euschistus variolarius* both the large and small idiochromosomes are distinctly rod-shaped, and at the late first prophase and metaphase it can be clearly demonstrated that the *large rod divides longitudinally and the small rod transversely*. There are rare (perhaps only apparent) exceptions to this, but as a rule one divides transversely *just as surely and as constantly* as the other divides longitudinally, and this we have demonstrated in a number of photographs.

The germ cells are being studied in order to obtain a morphological basis for conclusions or to put conclusions to a morphological test, and the idiochromosomes seem to offer a fruitful test of the theoretical value of longitudinal and transverse divisions. In order to retain our faith in the special significance that has been attributed to the longitudinal and transverse divisions we are driven to the inconsistency of accepting the evidence in the case of one idiochromosome, and disclaiming it for the other — though the evidence in both cases is equally strong. If, on the other hand, we accept the evidence and at the same time retain faith in the theory, we are forced to admit that the sister cells of the first division are also dimorphic and we have a so-called reduction division of one of the idiochromosomes of the first spindle as surely as we have a reduction division (the separation of the two idiochromosomes) in the second spindle, we thus have a somewhat similar phenomenon occurring in both spindles.

We have a number of photographs demonstrating both spermatocyte divisions and also a large number showing the spermatogonial, oögonial and embryonic chromosome groups — several hundred in all, but the publication of these must be reserved for a separate paper, where we shall aim to make a very full comparison of these chromosomes, giving especial attention to the groups that have not been presented in the papers which advocate the sex-determination hypothesis. We believe that these groups are of special value — that we have no right to ignore exceptions because they do not fall in line with certain theories. The trite claim that exceptions prove the rule loses its force when enough exceptions are found to challenge the rule. A careful examintion of our preparations makes it possible to select chromosome groups which exactly fit a given theory, but many groups can also be found that are a serious menace to these theories, while on the other hand they present no difficulties to the conception of those who regard the number, size and form of the chromosomes as inherited characters — the expression of cell activities rather than the cause.

January, 1909.

Appendix.

We have just received Wilson's latest "Study on Chromosomes," No. V.,[1] in which (in a footnote on page 159) he attempts to explain what he calls our "entirely mistaken conclusions" regarding the division of the accessory chromosome in *Anasa tristis* as follows:

"The regrouping of the chromosomes in the second division, first described by Paulmier ('99) in *Anasa tristis*, is characteristic of the Coreidæ generally, an eccentric position of the idiochromosome being a nearly constant feature of the first division but not of the second. Failure to recognize this fact in the case of *Anasa tristis* seems to have been one of the main sources of error in the entirely mistaken conclusions of Foot and Strobell ('07*a*, '07*b*) regarding this species. (Cf. Lefevre and McGill, '08.) Demonstrative evidence on this point is given by polar views of rather late anaphases, in which every chromosome of each daughter plate may be seen, in the same section. Such views, of which I have studied many, both in *Anasa* and other genera, show that one of the chromosomes may indeed occupy an eccentric position, and may there divide; but in such cases the odd chromosome is always found elsewhere in the group, lying either in or near one of the daughter groups and not in the other. When the odd chromosome is eccentric it is found in one of the daughter groups but not in the other."

In order to speak with authority on the regrouping of the chromosomes, some accuracy at least is expected in the identification of the individual chromosomes. Paulmier's authority can be dismissed when we recall Wilson's interesting discovery that in *Anasa tristis* Paulmier erroneously identified the microchromosome as the accessory (undivided) chromosome of the second spindle.

In Plate III. of our "Study of Chromosomes in the Spermatogenesis of *Anasa tristis*" ('07) we *show* in Photos 27, 28, 33, 35, 36, 37, 38, 39, 42, 43, 44 and 45, late anaphases or early telophases of second spindles demonstrating the ring-like formation of the chromosomes, with one of the chromosomes eccentrically placed or lagging. In our smear preparations this grouping is characteristic of the second division as it is of the first, although, as we have already pointed out, there are exceptions to this rule. There can be no possible doubt of our identification of these groups as second spindles, because they are invariably found in regions of the testis surrounded by spermatids in different stages of development and, further, the form of the chromosomes is so beautifully demonstrated in our smear preparations, it is hardly possible to confound the first and second spindles,[1] a mistake easily made in sections. That the individual chromosomes may change their relative positions at this stage, while the form of the group remains unchanged, is a possibility that any observer would naturally consider. But as we find in our preparations, the ring-like grouping of the chromosomes of the first spindle so constantly duplicated in the second spindle, with the microchromosome (the one chromosome that can be identified beyond question) maintaining its characteristic position in the center of the ring, the identification of the eccentric chromosome of both spindles as *probably* the same chromosome, is certainly as legitimate a conclusion, as forcing an arbitrary contradiction of this assumption, in order to support a theory.

---

[1] *Jour. Exp. Zoöl.*, Vol. VI., No. 2, 1909.

In complete opposition to what Wilson calls his "demonstrative evidence" on this point, we refer to our published photographs, Plate III. (in the paper quoted above), 29, 32, 33, 34, 35, 36, 37, 40, 41, 43, showing polar views of late anaphases or telophases of second spindles, in which every chromosome of each daughter plate can be counted. In all these preparations there is an eccentric, lagging — in most cases dyad — chromosome, but as *ten* chromosomes can be clearly counted at each pole, we fail to see how the situation is improved, for Wilson to claim, that where the lagging chromosome divides in this spindle it is not to be identified with the "odd" chromosome — which he says, "is, in such cases, *always to be found elsewhere in the group.*" We have given demonstrative evidence that this is not so in our preparations.

The essential fact is not the relative position of the accessory chromosome in either the first or second spindles, but whether one of the chromosomes consistently fails to divide in the second spindle. It has been said of the accessory chromosome that in the spindle in which it is heterotropic, it tries to divide, but does not succeed. In the case of *Anasa tristis*, we have demonstrated that it is by no means always unsuccessful in these efforts.

---

[1] The ring-like grouping of the chromosomes is also characteristic of the first and second spindles of *Euschistus variolarius*, where the distribution of the unequal tetrad in the second spindle adds conclusive proof of the identity of the spindles.

We regret that we cannot agree with Professor Wilson as to the value of the support he believes he has received from Lefevre and McGill's paper on *Anasa tristis* and *Anax junius* ('08). In this paper our results in *Anasa* are summarily disposed of in two pages of curt contradictions, supported by ten sketches made from sections. The futility of such methods as opposed to the very full demonstration we have given in 107 photographs to support our conclusions in *Anasa*, must be apparent to any unprejudiced observer.

In this same paper, McGill corrects her earlier observations on *Anax junius* on three very important points:

1. In her first work on this form ('04) (in which she expresses her indebtedness to Professor Lefevre) she found no evidence of a chromatin nucleolus in the resting spermatocyte. She and Lefevre now find, *in the same material*, a chromatin nucleolus persisting through the resting spermatocyte.

2. In her earlier work McGill identified the microchromosome as the accessory chromosome. She and Lefevre now identify, *in the same material*, one of the larger spermatogonial chromosomes as the accessory chromosome.

3. In her original count of the spermatogonial chromosomes, McGill found an even number, 28. She and Lefevre now find *in the same material* an odd number, 27 spermatogonial chromosomes.

In view of these contradictions we may justly hesitate to accept as definitive the recent conclusions reached by McGill and Lefevre in *Anasa tristis*, believing a new point of view may give us still further variations in their very interesting observations.

BIBLIOGRAPHY.

Arnold, George.
'08 The Nucleolus and Microchromosomes in the Spermatogenesis of Hydrophilus piceus (Linn.). Arch. f. Zellforschung, Bd. II., Hft. I.

Deton, Willy.
'08 L'étape Synaptique dans l'ovogénèse du Thysanozoon Brocchii. La Cellule, t. XXV.

Fick, R.

'07 Vererbungsfragen, Reduktions und Chromosomen-hypothesen. Ergebnisse der Anat. u. Entwick-geschichte, Bd. XVI., 1906.

Foot and Strobell.

'05 Prophases and Metaphase of the first Maturation Spindle of Allolobophora fœtida. Amer. Jour. Anat., Vol. IV., No. 2.

'07 A Study of Chromosomes in the Spermatogenesis of Anasa tristis. Amer. Jour. Anat., Vol. VII., No. 2.

Häcker, Valentin.

'07 Die Chromosomen als angenommene Vererbungsträger. Ergebnisse u. Fortschritte der Zoologie, Bd. I., Hft. I.

McClung, C. E.

'02 The Accessory Chromosome — Sex Determinant? Biol. Bull., Vol. III., No. 2.

Meves, Fr.

'02 Struktur und Histogenese der Spermien. Ergeb. der Anat. u. Entw.-geschichte, Bd. XI., 1901.

'08 Es gibt keine parallele Konjugation den Chromosomen. Arch. f. Zellf., Bd. I., Heft. 4.

Montgomery, Th. H.

'98 The Spermatogenesis in Pentatoma up to the Formation of the Spermatid. Zool. Jahrb., Bd. XII.

'01 A Study of the Chromosomes of the Germ Cells of Metazoa. Trans. Amer. Philos. Soc., Vol. XX.

'06 Chromosomes in the Spermatogenesis of the Hemiptera heteroptera. Trans. Amer. Philos. Soc., Vol. XXI.

Moore, J. E. S., and Robinson, L. E.

'05 On the Behavior of the Nucleolus in the Spermatogenesis of Periplanata Americana. Quart. Journ. Mic. Sci., Vol. XLVIII.

Schäfer, Friedrich.

'07 Spermatogenese von Dytiscus. Zool. Jahrb., Bd. XXIII., Hft. 4.

Stevens, N. M.

'05–'06 Studies in Spermatogenesis. Publication No. 36, Parts I. and II., Carnegie Institution of Washington.

v. Tellyesniczky, Koloman.

'07 Die Entstehung der Chromosomen. Evolution oder Epigenese? Urban & Schwarzenberg, Berlin.

'05 Ruhekern und Mitose. Arch. f. mik. Anat., Bd. 66.

Wassilieff, A.

'07 Die Spermatogenese Von Blatta germanica. Arch. f. mik. Anat., Bd. 70.

Wheeler, William Morton.

'97 The Maturation, Fecundation and Early Cleavage of Mysostoma glabrum Leuckart. Archive de Biologie, Tome XV.

Wilson, E. B.

'05 Studies on Chromosomes, I. Journ. Exper. Zoöl., Vol. II., No. 2.

'05 Studies on Chromosomes, II. Journ. Exper. Zoöl., Vol. II., No. 4.

Explanation of Plates.

All the photographs were taken with a Zeiss Apo. 2 mm. immers. lens, 140 apr. and compensating ocular 4. Photos 1 to 31 are from smear preparations stained with a saturate solution of Bismarck brown. In all these preparations the *whole nucleus* is shown.

Magnification of Photos 1 to 10 inclusive, 1,000 diameters.

Magnification of Photos 11 to 32 inclusive, 600 diameters.

As some of the germinal vesicles were too large to be included in the field at a magnification of 1,000 diameters, we used a magnification of 600 diameters for all the oöcytes, in order to facilitate a comparison.

The reproductions were made by the Rotograph Company from our own negatives.

Plate I.

Euschistus variolarius.

Photo 1. Two young resting spermatocytes, first order, one showing two nucleoli and the other one nucleolus.

Photo 2. A little later stage than Photo 1. Two nucleoli are shown, unequal in size, but this inequality is not so marked as in the two nucleoli of Photo 6.

Photo 3. Resting first spermatocyte about the same stage of development as Photo 2. One nucleolus present.

Photo 4. A resting first spermatocyte showing two nucleoli about equal in size.

Photo 5. A resting first spermatocyte with two nucleoli, unequal in size, though the inequality here is not so conspicuous as in Photo 6.

Photo 6. A later stage than Photo 5. The chromatin shows the segregation which precedes the formation of the chromosomes. Two nucleoli are present, very unequal in size.

Photo 7. First spermatocyte with the chromatin somewhat more closely segregated than in Photo 6. One nucleolus is present showing a typical vacuole.

Photo 8. A larger first spermatocyte with one nucleolus, the disposition of the chromatin suggesting a network.

Photo 9. A first spermatocyte showing an early stage in the formation of the chromosomes ; one nucleolus is present.

Photo 10. A much later stage than Photo 9. The bivalent chromosomes are formed, but the number is not so clearly demonstrated as at later stages. (These later stages, as well as the transitional stages, between Photos 9 and 10, are reserved for a subsequent publication.) The nucleolar-like thickening in the loop of the chromosome on the lower periphery of the group is not a small nucleolus. In the preparation it can be plainly recognized as a segregation of chromatin at the point where a loop of the chromosome brings two parts of the thread in contact. Similar, though less conspicuous thickenings are seen at other points where two threads cross. One clear nucleolus is present in this spermatocyte.

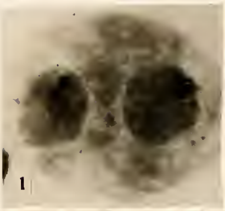
Photo 11. Nucleus of a young oöcyte, with the chromatin granules quite evenly distributed throughout the nucleus. No nucleolus present.

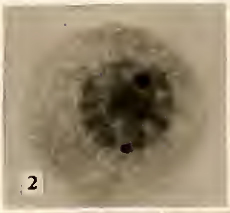
Photo 12. A slightly older germinal vesicle, with the chromatin segregated into clumps, too numerous to represent individuual chromosomes.

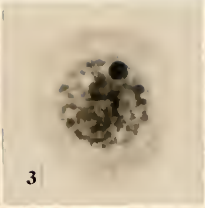
Euschistus variolarius.

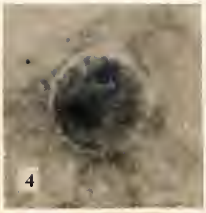
PLATE I

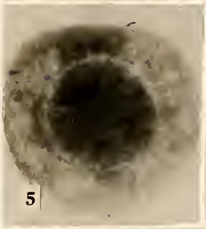
FOOT & STROBELL PHOTOS.

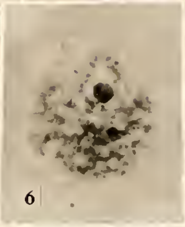

1

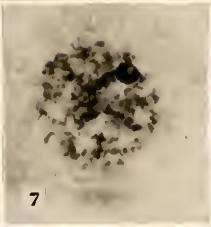

2

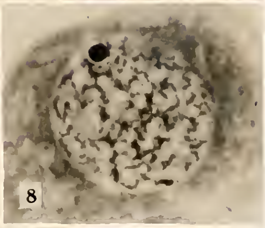

3

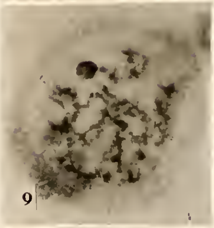

4

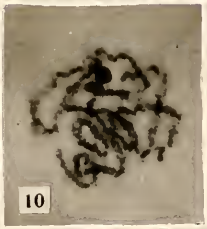

5

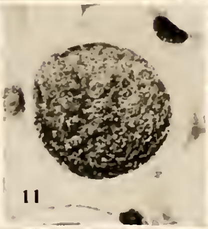

6

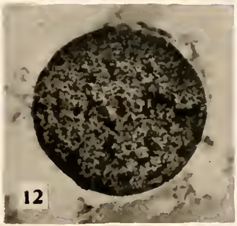

7

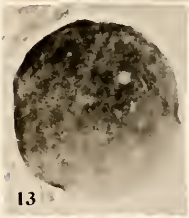

8

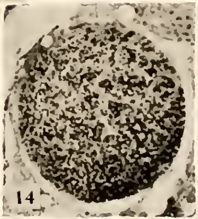

9

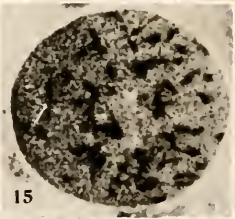

10

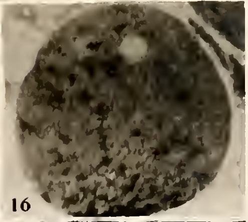

11

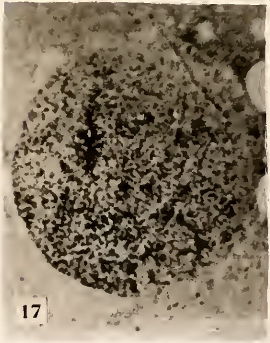

12

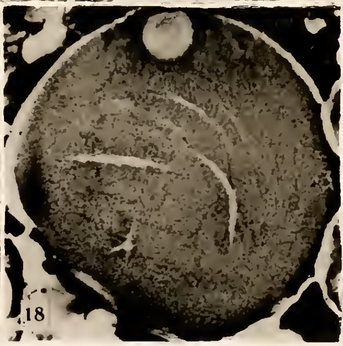

13


14


15


16


17


18

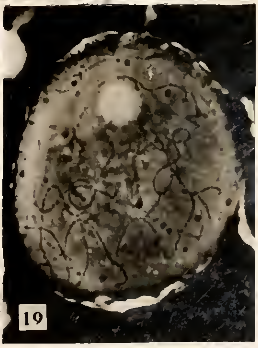

19

PHOTO 13. A young germinal vesicle with the chromatin almost in a diffused condition. A small, clear, spherical space is present, which may represent the first appearance of the achromatic nucleolus so conspicuous in the older germinal vesicles of Photos 18 to 30.

PHOTO 14. A young germinal vesicle with the chromatin evenly distributed as in Photo 11.

PHOTO 15. An older germinal vesicle with the chromatin segregated into numerous clumps as in Photo 12. Many of the clumps in this germinal vesicle appear almost as dense as nucleoli, though in the preparation they are seen to be merely a close segregation of fine granules.

PHOTO 16. An older germinal vesicle showing less pronounced segregations of chromatin, and a clear spherical space which can surely be interpreted as an early appearance of the achromatic nucleolus seen in the germinal vesicles of Photos 18 to 30.

PHOTO 17. A much larger germinal vesicle with the chromatin quite evenly distributed as granules.

PHOTO 18. An older germinal vesicle with the first indication of the formation of delicate chromatic threads, which later develop into the chromosomes. At this stage the achromatic nucleolus is always present. The cracked spaces shown in this germinal vesicle and in Photos 21, 23, 24 and 29 are due to uneven shrinkage as the germinal vesicles rapidly dry on the slide.

PHOTO 19. A much later stage than Photo 18 — the chromatin threads being well formed. (The next stage to Photo 18 is shown in Photo 20, Plate II.) The germinal vesicle of this preparation is not well flattened and some of the chromatic threads are very much out of focus.

Plate II.

Euschistus variolarius.

Photo 20. A germinal vesicle a little further developed than the one shown in Photo 18. The delicate chromatic threads are a little more defined. The achromatic nucleolus is present near the periphery.

Photo 21. A germinal vesicle with the delicate chromatic threads well formed. The achromatic nucleolus is seen on the periphery.

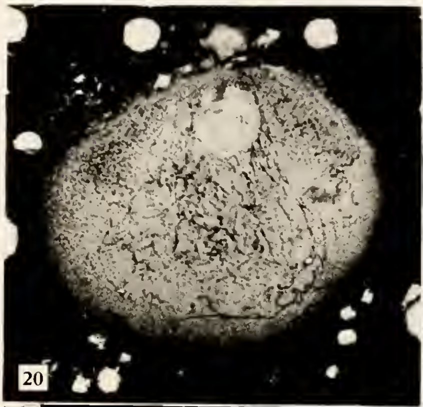
Photo 22. A germinal vesicle with the chromosome threads further developed. The nucleolus near periphery.

Photo 23. A germinal vesicle with long, thin chromosome threads. The nucleolus on periphery.

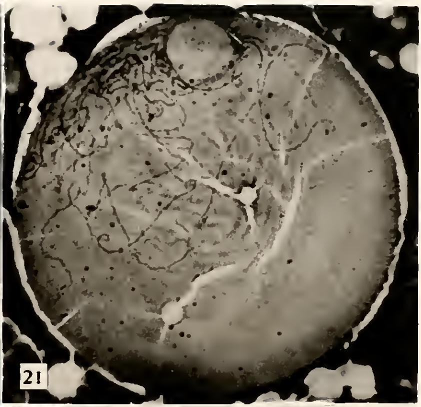
Photo 24. A germinal vesicle at about the same stage of development as Photo 23 The nucleolus on periphery.

Photo 25. A germinal vesicle with the chromosome threads further developed.

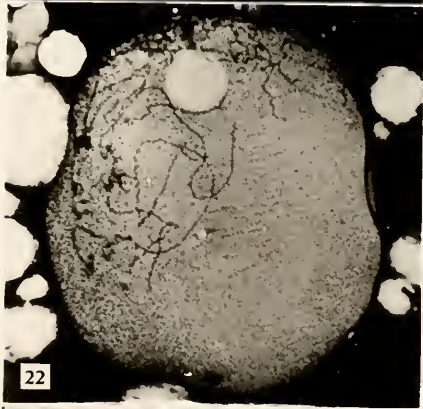
FOOT & STROBELL PHOTOS.



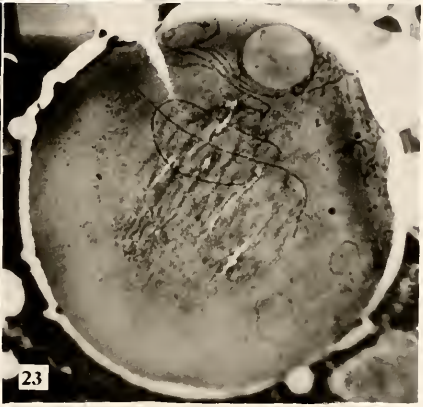
20



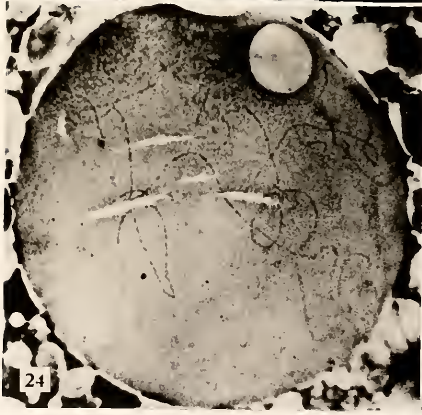
21



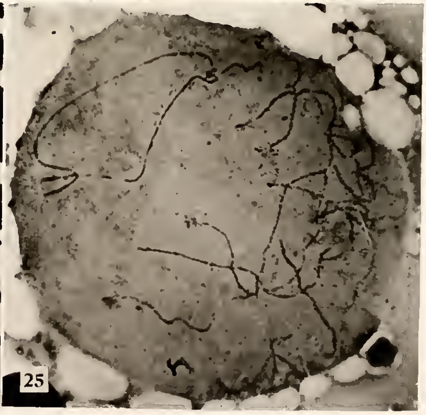
22



23



24



25

Plate III.

Euschistus variolarius and Allolobophora fœtida.

Photo 26. A germinal vesicle with chromosome threads well formed. Nucleolus on periphery.

Photo 27. A germinal vesicle at about the same stage of development as Photo 26. The nucleolus near the center.

Photo 28. A germinal vesicle with long, thin, twisted chromosome threads. Nucleolus near the center.

Photo 29. A germinal vesicle showing a later stage of development. The chromosome threads have contracted into shorter, thicker pieces. Nucleolus still present.

Photo 30. A germinal vesicle with the chromosomes almost at first prophase stage. Nucleolus still persisting.

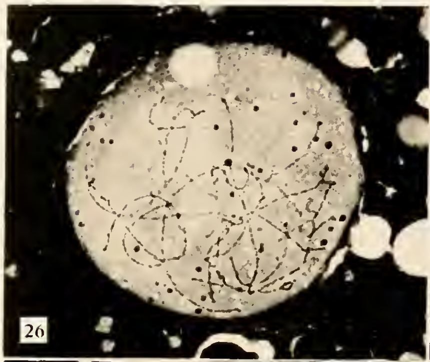
Photo 31. A germinal vesicle of *Allolobophora fœtida*. The same technique as that used for the *Euschistus* germinal vesicles (Photos 18 to 30). The chromosome threads are long, and thin, and longitudinally split. The large, less chromatic nucleolus is on the periphery, and the dense chromatin nucleolus is near the center.

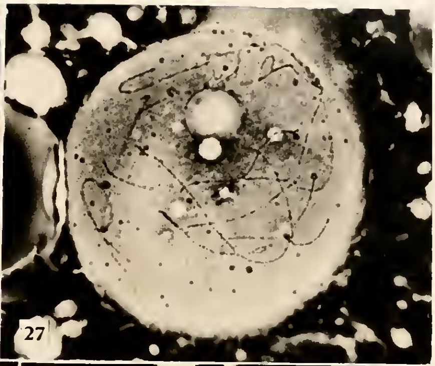
Photo 32. A section ($2\frac{1}{2}$ μ thick) through the first maturation spindle of *Allolobophora fœtida*. At the equator one chromosome is shown, the ten remaining chromosomes being clearly demonstrated in the adjoining sections. Near the upper pole we see the persisting chromatin nucleolus. A centrosome is shown at each pole

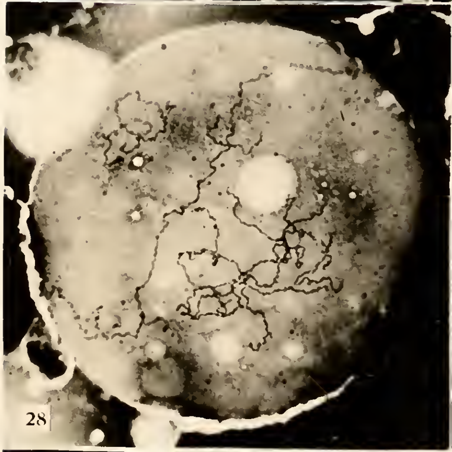
Euschistus variolarius.

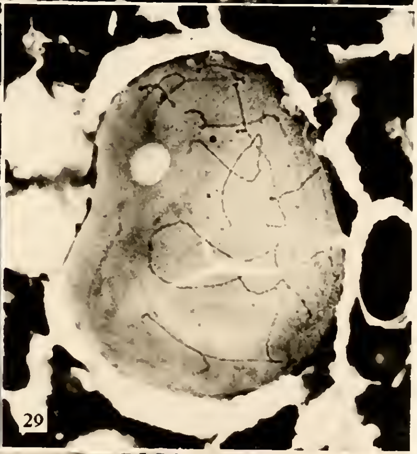
PLATE III

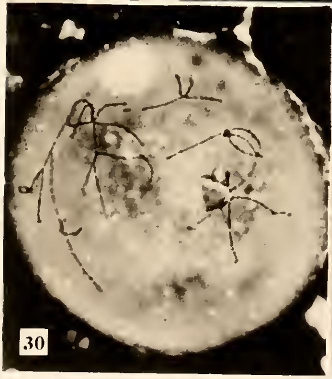
FOOT & STROBELL PHOTOS

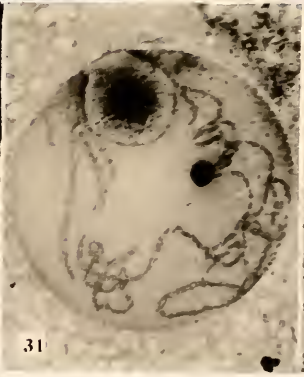

26

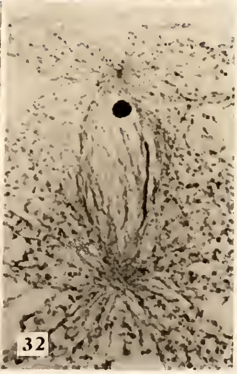

27


28


29


30


31


32

INHERITANCE IN THE "WALKING-STICK," APLOPUS MAYERI.

CHARLES R. STOCKARD.

The family Phasmatidæ, as is well known, shows some of the most striking cases of "protective resemblance" found among the insects. Several of the genera are typically stick-like to a surprising degree while members of the genus *Phyllium* resemble in detail a leaf-like structure. These animals are no doubt protected by their imitative forms provided they behave in a certain manner. In fact the protection or concealment of such an animal depends as largely on its behavior as upon its resemblance to surrounding objects. In order to ascertain whether these so-called protectively adapted insects really exhibited a "protective behavior" I[1] studied the habits of the "walking-stick," *Aplopus mayeri*, which is abundant on the Tortugas Islands, Florida. These large insects were found to behave in a manner almost ideal for their concealment among the twigs and stems of the plant on which they feed, *Suriana maritima*.

My study was made during a season, June and July, when the enemies of *Aplopus* were extremely rare on these islands. In the spring and fall, however, the great numbers of migrating birds which stop here no doubt devour many of these large Orthoptera in spite of their almost perfect concealment. But for their protective resemblance and habits birds might easily exterminate such slow-moving flightless insects within a few seasons, in fact the existence of creatures like *Aplopi* on these small islands is really dependent upon their ability to be passed unobserved by birds migrating between the eastern United States, West Indies and South America.

The question arises whether the protective behavior in *Aplopus* is fully developed on hatching from the egg or whether it is attained with their large size and mature condition. In order to investigate this and the further question of first-leg form considered below eggs were collected during the summer and brought to New York where the newly hatched individuals might be observed.

---

[1] "Habits, Reactions and Mating Instincts of the 'Walking-Stick,' *Aplopus mayeri*," *Science*, N. S., XXVII., 1908 — Publication No. 103, Carnegie Institution, Washington, 1908.

These eggs were kept in small loosely stoppered bottles in the laboratory at ordinary room temperature. They began hatching about the first of December and during January a large number of the insects came out.

Behavior of Newly Hatched Aplopi.

The reactions of the small insect on the day it emerges from the egg are almost identical with those of the fully mature eight-inch females which I studied at the Tortugas. The young *Aplopi* are a light chocolate-brown in color with yellowish bands about the legs and the sexes are similar. The adult males, however, become greenish in color while the female retains her original brown. The adults also have rudimentary wings which are capable of being raised when the insect is excited, but the young are wingless. Their reactions will be referred to briefly at this time as they are given in some detail in my former paper.

The insects when at rest among the twigs assume an attitude which in consequence of their stick-like shape makes them most difficult to detect. The first pair of legs are stretched directly forward enclosing the head between thin curved portions of the femora which fit perfectly against it. The antennæ are brought

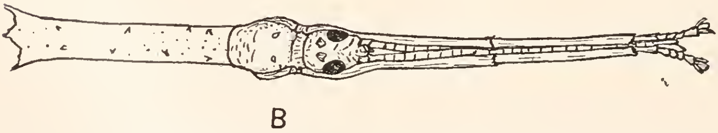


Fig. 1. *A*. Lateral aspect of *Aplopus*, head and first two thoracic segments. The first pair of legs are stretched forward in the typical resting attitude. The femur fits perfectly about the ventro-lateral region of the head and leaves the eyes uncovered.

B. Dorsal view of the same specimen showing the approximated antennæ directed forward and enclosed between the first pair of legs.

together between the first legs, Fig. 1, *B*. (See also Fig. 1, Plate I., and the photographs in my former paper.) The anterior end of the insect thus closely resembles a more or less pointed stick. The newly hatched individual habitually assumes this position and the point of especial interest, which I shall return to later, is that the thinned out curved part of the femora fit about the head as perfectly the first time the legs are stretched forward as they do in the adult.

In walking from place to place the adult moves slowly and often exhibits a slow laterally swinging motion suggesting a twig swinging in a light breeze. The young also swings its body from side to side in a similar manner. When a number of young *Aplopi* are sitting motionless if the observer blows a current of air over them they all begin to swing very actively from side to side as if being swung by the breeze. This swinging motion no doubt serves to render them less conspicuous among the shrubs.

The newly hatched individuals use the same methods to escape an enemy as those employed by the adult. When they are touched or pinched slightly they move away a short distance and immediately come to rest again, if the stimulus be repeated they begin to walk at a more rapid gait than before and move a greater distance away. If again touched they drop bodily to the floor and feign death just as the adult does. The death-feigning reaction is more readily induced in the young than in the adult, and no doubt serves to great advantage in enabling them to escape an enemy which fails to seize them securely in the first attempt. The chances of escape for this stick-like creature when it drops through the dense foliage and branches of the *Suriana* bush is most favorable. When in the death-feint the legs may be bent in any position and the body twisted without the least move on the part of the animal. They may actually be piled one on another and will remain as motionless as dead insects.

The young walking-stick crawls upwards on any object that it may reach after emerging from the egg. As I previously recorded the female *Aplopus* sits in the *Suriana* bushes and lays its eggs which fall to the ground where they later hatch. Thus the tendency of the young to crawl upwards on the first object with which it comes in contact serves to bring it up the *Suriana* bush to its leafy food. In crawling up the young insect waves its antennæ to feel the way just as does the adult and reaches out with the first legs to grasp the object located by the antennæ.

Finally the young like the adult is more or less nocturnal in its movements. During the day they sit motionless with the first legs extended forward but at night they become active and move about to feed. The food of the adult is limited to the leaves of *Suriana*. I have made no attempt to feed the young since they may be kept alive for about one week after hatching without taking food.

THE THIN CURVE OF THE FEMORA WHICH FITS AGAINST THE VENTRO-LATERAL SURFACES OF THE HEAD.

When the first pair of legs are extended forward the femur of each is so curved near its proximal joint as to fit perfectly against the ventro-lateral parts of the head and at the same time leaves the eyes uncovered, Fig. 1, *A* and *B*. The curved portion of the femur is also very thin in a lateral direction and thus when pressed closely to the head the legs go out as almost straight lines instead of bulging around the head to any great extent. It seems difficult to believe that the first pair of legs could through chance variations or mutations have come to fit so perfectly around the sides of the head and at the same time to have their dorsal line so curved as to leave the eyes uncovered. It must be remembered that when the first legs are in the extended position the head presses against the dorso-lateral surfaces of the femur and not straight against the inner lateral surface only. This arrangement may be better understood by a close examination of the dorsal and lateral views given in Fig. 1, *A* and *B*.

The possibility suggests itself that the perfection of the fit is attained during the life of the individual since the legs are so habitually pressed against the head for about twelve hours daily. To test this it became desirable to study newly hatched individuals in order to find whether the femur curve was as perfectly adjusted in them as in the adult. A careful examination of about one hundred *Aplopi* shortly after emerging from the egg has convinced me that the curve of the femur is as true to the head pattern in the newly hatched young as in the mature insect when several months old.

Finally, is this adjustment between the structure of the femora and head due to the position of the insect when enclosed within the inelastic egg shell? If the first legs were folded forward against the head the pressure during embryonic life might easily be sufficient to mold the femur curve into pattern for the head. Twenty eggs containing embryos at various stages shortly preceding hatching were dissected with this question in view. The elliptical egg shell is of a rigid chitinous material with a circular operculum at one end and a hilum-like scar on one side to which the inner egg membrane is attached. The size of the egg ranges from 2 mm. in narrow diameter by 4 mm. in long diameter to 3 mm. by 4.5 mm. The length of the walking-stick on hatching is from 17 mm. to 23 mm. measured from the tip of abdomen to tip of the first pair of legs when extended forward, or from tip of head to tip of tail 9.5 mm. to 13 mm. The embryo within the egg is, therefore, necessarily much folded and bent.

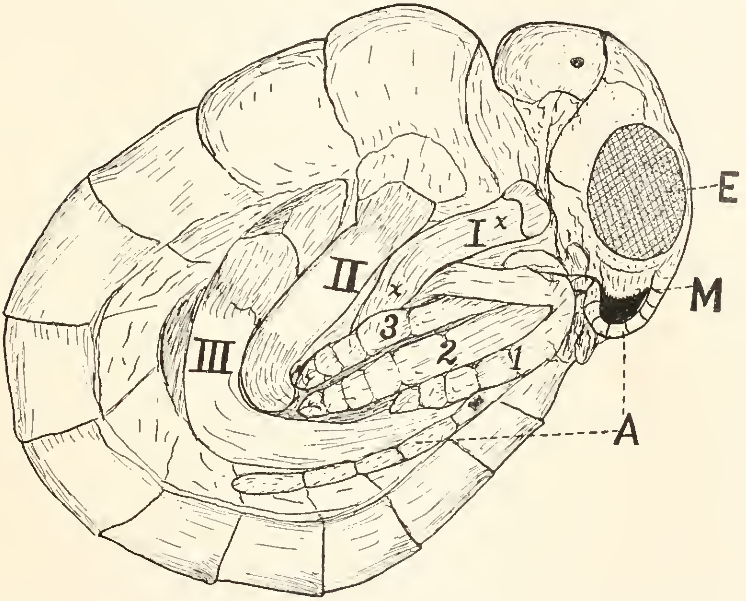


FIG. 2. An unhatched *Aplopus* with its egg membranes dissected away showing its folded position. The femora of the three right legs are marked with roman numerals and the tarsal ends of the same legs are indicated by figures. The part of the first femur which in later life fits against the head is shown between the points *x–x*, it is not molded against the head in the egg. *A*, antennæ; *E*, compound eye; *M*, mandible.

On dissecting the egg the embryo is found to be curled around in a rather constant manner, the head being usually, though not always, near the opercular end. The long legs are folded back and forth upon themselves in a very definite fashion as shown by the camera drawing, Fig. 2. The antennæ (*A*) pass down the front of the head and then back along the ventro-lateral surface of the abdomen being sometimes bent around the first pair of legs. The point of most importance is that the femoral segments of the legs are all directed obliquely away from the head. The first pair of legs each of which is folded on itself four times does not touch the sides of the head at all. The head and large eyes are entirely uncovered and exposed. The femora of the first pair of legs not only fail to mold their curves against the head but the femora are so pressed against the thorax that the surfaces which will subsequently be concave (in Fig. 2 between x and x) are actually arched convexly. Thus it is seen that the mechanical arrangement of the embryo's parts within the egg is not responsible for the fit of the femur curve against the head. On the contrary the curve seems to develop in spite of these arrangements.

When hatching the embryo's head and body come forth from the egg first, the antennæ are then pulled out, the legs being the last parts liberated from the shell, Fig. 3. It often happens that the shell is carried around for some time dangling to the third pair of legs. In Fig. 3 the well developed curves of the femora are distinctly shown, x to x, and are being pulled in a direction away from the head, yet as soon as the legs are free from the shell the first pair may be straightened forward and their curved femora fit neatly against the sides of the head. We see, therefore, that the curve of the femora to fit the sides of the head is a character transmitted to all of the young and perfectly formed at the time of hatching. It might seem that the origin of this character was most probably due to the habit of the insects to press the first pair of legs against the head. Gradually this pressure developed a thin concave region of the femur of the first leg which molded itself more and more perfectly to the contour of the head. If this curve arose in any other way the second and third pairs of legs might have developed at least a trace of such a character though this is not absolutely necessary. It must be remembered that the curves fit the ventro-lateral contour of the head to a remarkable degree.

When the first pair of legs are so stretched forward the insect's antennæ are brought together. The legs have an irregular groove extending along the approximated surfaces and when complete approximation takes place a rather imperfect tube is formed enclosing the antennæ. This is a case analogous to the above and it is difficult to imagine how chance variations could bring about such mechanical harmonies between organs only associated through an habitual attitude assumed by the animal when at rest. Yet it must not be forgotten that many other equally as nice morphological arrangements exist which have no habit or action connected with them. Indeed a crucial case of use inheritance is almost impossible to imagine from purely descriptive work. I would not be understood as advocating any principle of inheritance but merely bring forward the present case as being of interest in itself.

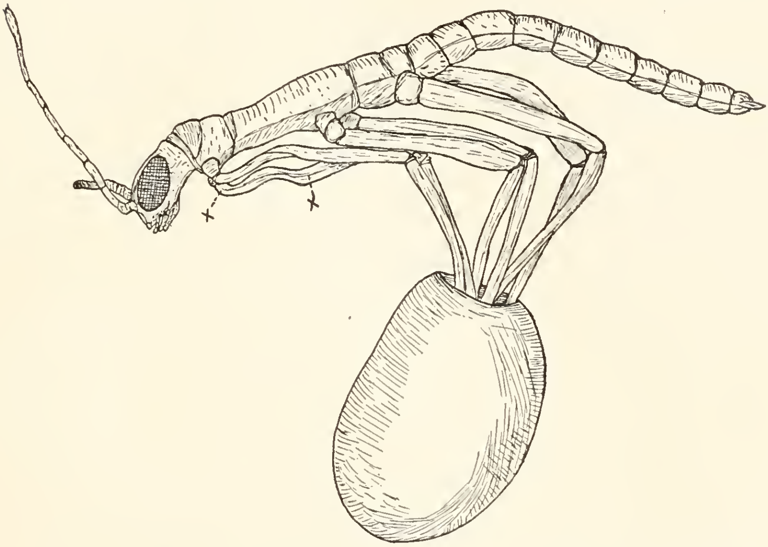


FIG. 3. *Aplopus* in the act of hatching from the egg. The body and head come out first, then the antennæ and finally the legs free themselves from the shell. The parts of the first legs between x–x are curved to fit the head when they are straightened forward although they have never touched the head up to this time.

CORNELL MEDICAL SCHOOL, NEW YORK CITY,
February 6, 1909.

THE CONNECTIONS OF THE GONADIAL BLOOD VESSELS AND THE FORM OF THE NEPHRIDIA IN THE ARENICOLIDÆ.

ELLIOT ROWLAND DOWNING, Ph.D.,

Northern State Normal School, Marquette, Michigan.

The gonads of *Arenicola* occur on certain blood vessels that lie diagonally on the exterior of the glandular portion of the nephridia and that are consequently designated the gonadial vessels. In a study of the gonads, the results of which will be published shortly, it was found that the literature of the subject contained conflicting and inaccurate statements regarding the relations of these gonadial vessels to their connecting vessels. This paper is the result of an attempt to determine these relations.

Gamble and Ashworth, in 1900, published an extended study of the anatomy of the several species which also reviewed the important literature on the family. They had previously published, 1898, a paper on the anatomy of *Arenicola marina.* In an article published in 1899, V. Willem disagreed with some of their statements as did R. Lillie in a more recent publication, 1906. These contradictory statements I shall try to adjust: I must also take exception to some other statements of each.

The general relations of the blood vessels may be easily understood from the accompanying partly diagrammatic figure of a cross-section at about the level of the first nephridium of *A. cristata* (Fig. 1). It will be noted that there are two main blood vessels, a dorsal and a ventral; these run the entire length of the animal. There is a pair of neurals of much smaller caliber, also extending the full length of the body. The paired gastric laterals and subintestinals, as also the paired integumentary vessels known as the parietal (or dorsal longitudinal) and the longitudinal nephridial, are limited in their extent and vary in length and prominence in the several species.

All previous authors agree in the arrangement of the nephridial vessels as follows: The afferent vessel, on approaching the nephridium, divides, one branch going to the setal sac and gill, if present, one to the integumentary vessels and one to the nephridium. The latter enters the nephrostome and forks, one branch traversing each lip. After reuniting, the vessel, reformed, runs out onto the wall of the nephridium as the gonadial vessel. Each of the three main branches of the afferent vessel gives off smaller vessels which, with more or less anastomosing with adjacent vessels, form capillary net-works in the organs supplied.

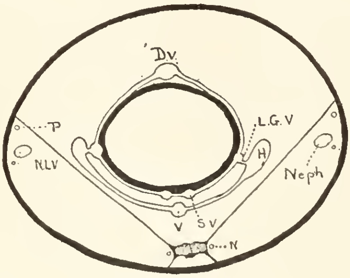


FIG. 1. Partly diagrammatic cross-section of *Arenicola cristata* at about the level of the first nephridium. *D. V.*, dorsal blood vessel. *H.*, heart. *L. G. V.*, lateral gastric blood vessel. *N.*, neural blood vessel. *N. L. V.*, nephridial longitudinal blood vessel. *P.*, parietal blood vessel. *S. V.*, sub-intestinal blood vessel. *V.*, ventral blood vessel.

The efferent vessels are formed by the union of capillaries in these same regions.

The afferent vessel, as a rule, comes from the ventral vessel. This is true for all except the first two nephridia of *A. Grubii* and of *A. ecaudata*, which nephridia are supplied, the first by a branch of the dorsal, the second by a branch of the parietal vessel (Fig. 2, *d*). In addition to the afferent vessel from the ventral vessel, the following nephridia also receive branches from the dorsal vessel: the first of *A. cristata*, the first two of *A. Claparedii* and the first three of *A. marina* (Fig. 2).

The following nephridia return blood directly to the subintestinal vessels through efferent vessels whose numerous branches are adjacent to their funnels: the fourth, fifth and sixth of *A. cristata*, the fourth and fifth of *A. Claparedii* and the fifth and sixth of *A. marina*. All others must pour the blood into the parietal and nephridial longitudinal vessels which, in turn, pass it to some of the more posterior efferent vessels (Fig. 2).

These observations are directly antagonistic to those of other investigators on several points of anatomical detail and of function of some of the vessels. I have given above a statement of the general arrangement of the branching afferent vessels with reference to the nephridia; it will be evident from text-figure 2 that my results only agree in a general way with the statements of previous authors. Thus in all nephridia of *A. Claparedii*, in some of *A. marina* and occasionally in *A. Grubii*, the afferent branch of the ventral vessel enters the *apex* of the funnel before it branches. In *A. cristata* one finds frequently, particularly in the somites containing the second and third nephridia, that the affer-

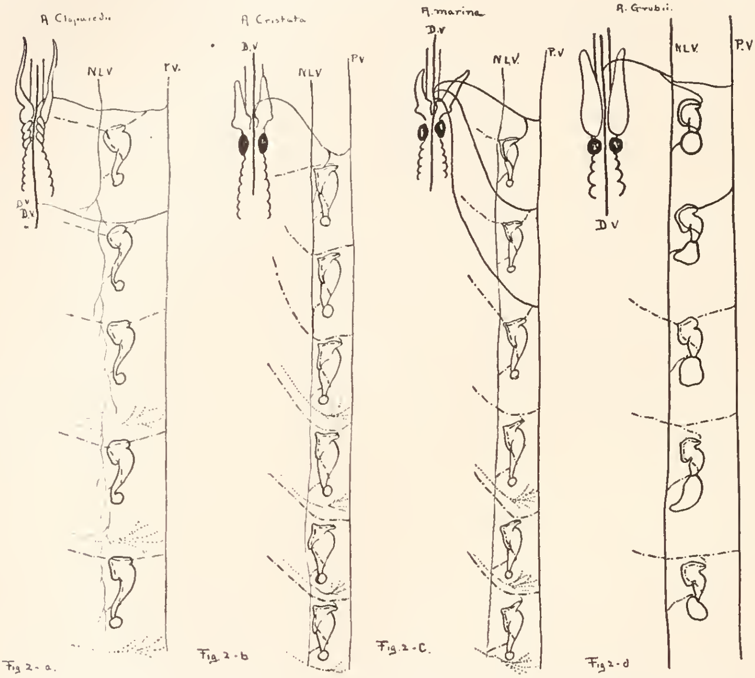


FIG. 2. Diagrams of the blood vessels supplying the nephridia in the several species of *Arenicola*, *a*, *A. Claparedii*, *b*, *A. cristata*, *c*, *A. marina*, *d*, *A. Grubii*; *d* will also serve as a diagram of the blood vessels of *A. ecaudata*. The form of the nephridia and of the digestive organs in the latter species, would be somewhat different but the arrangement of the blood vessels is the same as in *A. Grubii*. The dorsal vessel is shown by a solid line, thus ___. Afferent vessels arising from the ventral vessel are shown in broken lines, —.—.—. Efferent vessels connecting with the sub-intestinal blood vessel are shown in dotted lines, ... *D. V.*, dorsal vessel. *N. L. V.*, nephridial longitudinal vessel. *P. V.*, parietal blood vessel.

ent vessel gives off no branches to the nephridia, but the latter are supplied by a branch from the parietal.

The branch of the dorsal vessel to the second nephridium is small in *A. Claparedii*, but functional still. Gamble and Ashworth show and describe an afferent vessel to the first nephridium of *A. marina;* with this V. Willem disagrees, showing for this nephridium only a branch from the dorsal. In the majority of cases I find a branch from the ventral vessel also, thus agreeing with Gamble and Ashworth; in a few animals it is apparently wanting. I do not find, however, the efferent vessel from the fourth nephridium, in *A. marina*, to the subintestinal, which Gamble and Ashworth show but which V. Willem claimed was not present.

It is not true that the three main branches of the afferent vessel break up into capillaries; the one to the setal sac and gill does. The one to the integumentary vessels unites with them. The branch which enters the funnel continues as the gonadial vessel, runs peripherad to the nephridium and connects with the nephridial longitudinal, except in *A. Claparedii.*

The parietal and nephridial longitudinal vessels are distinct throughout the nephridial region in *A. Grubii*, *A. ecaudata* and *A. marina.* In *A. cristata*, the parietal is distinct but the nephridial longitudinal, while large at the level of the first nephridium, tapers posteriorly, becoming obscure back of the third nephridium. The parietal vessel is distinct the entire length of the nephridial region in *A. Claparedii.* The statement of Gamble and Ashworth that it is absent in this form is only explicable because they worked on preserved material; it is plainly evident in the living worm. The nephridial longitudinal, as a distinct vessel, is absent in this form except in the region of the first nephridium: its place is taken by a series of small connecting vessels, as if the gonadial vessel branched and some of its branches ran back to connect with those of the next posterior gonadial vessel.

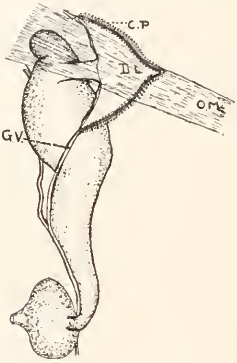
In all cases the branches of the dorsal vessel running to the nephridia are afferent vessels. Gamble and Ashworth state that "The first nephridia of A. Grubii and A. ecaudata are *supplied* by a branch from the dorsal vessel" (the italics are mine), yet "The first three nephridia of A. marina, the first two of A. Claparedii and the first of A. cristata *return* blood to the dorsal vessel." On *a priori* grounds, it would be strange to find the homologous blood vessels in closely related species carrying blood in opposite directions. I am certain that in *A. cristata* the blood flows from the dorsal vessel out to the parietal, the nephridial longitudinal vessel and to the nephridium, in this branch of the dorsal vessel. In this species the individuals are so large that in chloretonized specimens the direction of the blood movement is seen with comparative ease. S. E. Keith who has worked carefully for some time on the circulation of *A. cristata* gives me permission to quote her on this point as follows: "I have referred to these blood vessels in my own notes as the third pair of branches of the dorsal vessel. Gamble and Ashworth speak of the dorsal as receiving these branches, but the blood flows outward in them I am sure."

I have watched the blood-flow in *A. Claparedii;* the skin is frequently so transparent it may be seen without dissection. The flow is certainly away from the dorsal vessel in this species also. I am reasonably certain that such is the case in *A. marina,* in chloretonized specimens of which I have watched the blood movement. In this opinion I am supported by Willem who says of *marina,* "Il faut remarquer de plus, au point fonctionnel, que le sang circulant dans le tronc dorsal d'arriere en avant, le contenu des trois branches qui en emanent progresse dans une direction centrifuge."

Lillie makes the statement that "The vessel (segmental) begins its formation at the junction with the subintestinal blood vessel. Near its junction with the body wall the main vessel (segmental) gives off a branch (the nephrostomial vessel) which curves back, passes inward and barkward along the dorsal lip of the nephrostome." From this quotation it is evident that Lillie traces the afferent nephridial vessel to the subintestinal blood vessel, while, as stated above, I trace it, in agreement with Gamble and Ashworth and other observers, to the ventral vessel. The contradiction is only verbal, not real, for Lillie, in the explanation of his figures, labels this subintestinal vessel "the subintestinal *or* ventral vessel," a usage which he gets from oligochæt anatomy and which is incorrect here. Lillie finds that in *Arenicola* as in other *Polychæta*, (see Fraipont on *Poygordius*, for instance), as well as in the *Oligochæta* (Wilson on *Lumbricus*), the first vessel to arise as a differentiation of the mesoderm, is the ventral vessel. Wilson says "The first vessel to appear (in *Lumbricus*) is the ventral or subintestinal." What the relation of this first vessel genenally is to the development of the circum-intestinal network in the polychæts is an unsettled point. (See Edward Myer's "Studien über den Körperbau der Anneliden," III., p. 464.) Recently Schiller writes as follows: "Nur bezuglich Blutsinus und Darmgefäsznetz ist mann bei *Arenicola Grubii* noch nicht ins klare gekommen, welches von den beider das primäre sei, da verscheidene Autoren ganz verscheidener Ansicht daruber sind. Die einer behaupten dasz zuerst der Sinus auftrete und sekundär sich in ein Netz auflöse: die anderen bezeichnen den Blutsinus als ein Produkt des zusammengeschmolzen Darmgefäsznetzes." He does not care to express an opinion on the subject although of the growing posterior region of *A. Grubii* he says: "Im Darmepithel keine besonderen Zellen vorkommen, die Antrit an der Bildung eines Sinus nehmen könnten." In considering der Blutsinus, Darmgefäsznetz und Subintestinalgefäsz he says: "Diese drei Gebilde werden hier zusammen als anatomisch und entwickelungsgeschichtlich zusammengehörende Elemente."

I have taken pains to quote in order to show that in spite of the difference of opinion regarding the origin of the network of intestinal vessels there is no tendency to include the ventral vessel in *Arenicola* or other polychæts as a part of this network which does, however, include the subintestinals. In *A. marina* we know (Benham, 1893) that the subintestinal vessels and the gastric longitudinals develop as the result of a fusion of the walls of the circular vessels of the alimentary canal. While in the oligochæts the subintestinal vessels split off from the ventral vessel. (Beddard, F. E., "Monograph of the Oligochæta," p. 70.) The term subintestinal vessel is an incorrect term for the vessels so named in *Arenicola*. Lillie should not have used the term subintestinal when he meant ventral, as these two terms are not interchangeable in *Arenicola*. The ventral vessel or subintestinal vessel of *Lumbricus* — they remain united in this form — is not homologous to the ventral plus the subintestinal vessels of *Arenicola*.

There is need of amendment in the description and figures of the form of the nephridia of the genus. The funnels, in particular, are much more symmetrical, in some species, than they have heretofore been shown. The usual technique is at fault in distorting them. The funnels are attached to the oblique muscles in *A. Claparedii*, *A. cristata* and *A. marina*. When the worm is opened and pinned out for dissection, the strain on these oblique muscles or on the connecting blood vessels of the other species, pulls the funnels throughly out of shape. It is, therefore, well to stupefy the worm by adding, drop by drop, seventy per cent. alcohol to the smallest quantity of sea water that will cover the worm in a long narrow dish. Then when the muscular walls are relaxed, the body cavity is injected by means of a hypodermic syringe or a fine pipette, until the walls are well distended with the fixing agent. (Kopsch fluid answers well.) The whole worm is then placed in the fixing fluid. The nephridia are thus hardened without distortion and when dissected out later show their proper form.



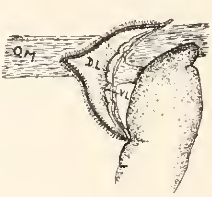
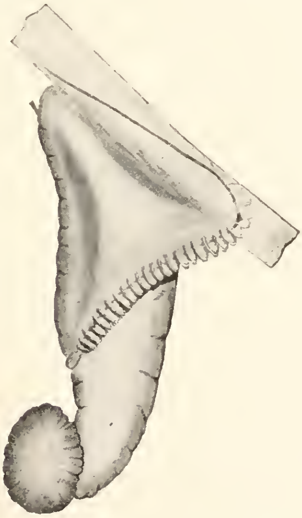


FIG. 3. Dorsal view of the second left nephridium of *Arenicola cristata*, × 3. *C.P.*, ciliated plates. *D.L.*, dorsal lip. *G.V.*, gonadial blood vessel. *O.M.*, oblique muscle.

FIG. 4. Ventral view of the anterior portion of second left nephridium of *Arenicola cristata*, × 3. *D.L.*, dorsal lip. *V.L.*, ventral lip.

The figures of the funnels or entire nephridia of each of the species are shown in the accompanying figures. The funnel of *A. cristata* (Figs. 3 and 4) is the most complicated and at the same time the most symmetrical in the genus. It is flattened, with a broadly sagittate or hastate form. Its dorsal lip, which is attached by its outer surface to the oblique muscle, forms the point of the arrow. The ventral edge of this lip is set with from thirty to sixty ciliated plates which stand nearly at right angles both to the plane of the lip and to its edge. A loop of the blood vessel which traverses the dorsal lip runs up into each plate. The ventral lip is bow-shaped and divided into three segments like the handle and ends of a bow; the convexity of the bow turns toward the apex of the dorsal lip. The opening of the nephrostome is a long narrow slit between the base of the triangular dorsal lip and the bow-shaped ventral lip; the opening leads into the rapidly narrowing throat. The hastate funnel is held to the body by a short slender haft; the body of the nephridium, the glandular portion, is club-shaped; the funnel attaches to its side near the larger end, the axis of the



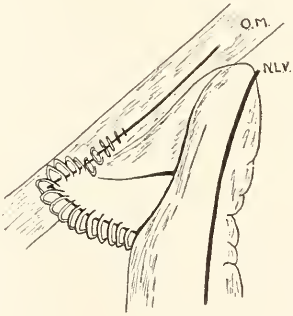
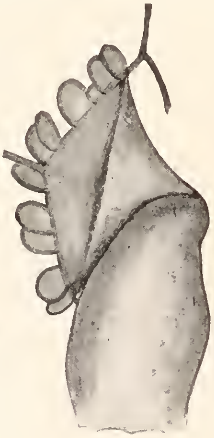


FIG. 5. Dorsal view of a nephridium of *A. marina*, × 15.

FIG. 6. Ventral view of the anterior end of the nephridium of *Arenicola marina*, × 15. *N.L.V.*, nephridial longitudinal blood vessel. *O.M.*, oblique muscle.

funnel being nearly at right angles to the longitudinal axis of the body. The body of the nephridium joins the roughly spherical bladder at the smaller end of the body, the handle of the club. The bladder opens to the exterior on its peripheral side by a very short duct leading to the nephridiopore.

The nephridium of *A. marina* approaches, at times, quite nea to the form of that of *A. cristata ;* but usually it is quite different from it (Figs. 5 and 6). The general shape of the body and of the bladder is nearly the same as in the nephridium of *A. cristata*, although in *A. marina* the body is more frequently curved while that of *A. cristata* is straight. The funnel shows great variation and often departs widely from the type of *A. cristata.* It is a flattened flap having roughly the shape of an equilateral triangle. At one angle the funnel opens into the anterior end of the body of the nephridium ; along the opposite side lies the nephrostome. One of the sides adjacent to the neck is attached to the oblique muscle ; the other adheres to the margin of the body. The axis of the funnel forms, therefore, an angle of only thirty or so degrees with the axis of the body. The dorsal lip is straight or slightly concave ; it is set with twenty five or thirty somewhat lanceolate ciliated plates, each of which is supplied with a loop of the blood vessel. The ventral lip, Fig. 6, is regularly concave.



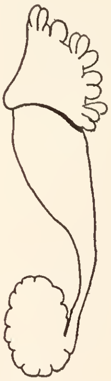
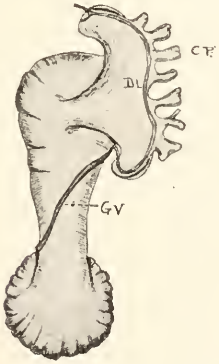


FIG. 7. Ventral view of the anterior portion of the third left nephridium of *Arenicola Claparedii*, × 30.

FIG. 8. Dorsal view of the nephridium of *Arenicola Claparedii*, × 15.

The ciliated plates which run along the edge of the dorsal lip, tend to continue along the blood vessel past the angle which the dorsal lip makes with the side of the funnel that attaches to the oblique muscle. This blood vessel runs along the muscle nearly parallel with the edge of the funnel. There are from ten to twenty of these plates ; those along the blood vessel on the muscle diminish in size. When occasionally there are an unusally large number of them along the blood vessel beyond the angle, the angle of the funnel adjacent to the muscle tends to become the apex of the funnel as in *A. cristata*, and the mouth shifts so as to more nearly face this angle instead of a side. In such cases the throat of the funnel opens into the body some distance back of the anterior end of the latter.

The funnel of the nephridium of *A. Claparedii* (Figs. 7 and 8) is least complicated. If we imagine a simple funnel form with short stem to be flattened and to have one lip pulled out into a triangular protrusion, we may gain a clear idea of the funnel of this species. The apex of the dorsal lip is broadly obtuse; the ventral lip is straight. The dorsal lip is set with the ciliated plates characteristic of the *marina* section of the genus which, as Gamble and Ashworth point out, includes the species *Claparedii*, *cristata* and *marina*. There are ten or twelve of these plates in *A. Claparedii.* The funnel is at the anterior end of the body and its axis is also at right angles to the axis of the body of the nephridium.



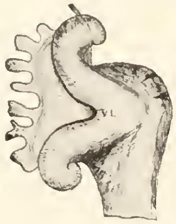


FIG. 9. Dorsal view of the first left nephridium of *Arenicola ecaudata*, × 15 *C.P.*, ciliated processes. *D.L.*, dorsal lip. *G. V.*, gonadial blood vessel.

FIG. 10. Ventral view of the anterior portion of nephridium (first left) of *A. ecaudata*, × 15. *V.L.*, ventral lip.

The funnel of the nephridium of *A. ecaudata* (Figs. 9 and 10) is reniform in outline with revolute ends as seen from the ventral face. It also is flattened; the dorsal lip is slightly concave, the ventral lip is deeply indented. The dorsal lip is provided with ten to thirty blunt, at times much branched, finger-like processes;

these are covered with cilia, and within each is a blood sinus instead of a loop of the blood-vessel. The throat of the funnel is relatively wide; the funnel attaches at the end of the body and usually has the customary position with its axis at right angles to the long axis of the nephridial body. Not infrequently however, the axis of the funnel is continuous with the axis of the body and we have a simple, unbent, tubular nephridium (Fig. 11). It is interesting to find such variation in this species for it makes evident how the more aberrant forms of the nephridium, such as occur in *A. marina*, are derived from a simple tubular type; just as a paper tube may be bent with its end at right angles to its major axis.

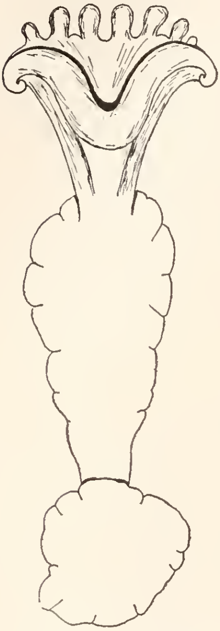
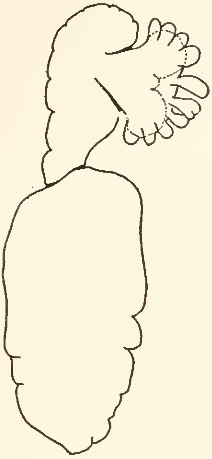


FIG. 11. An occasional form of the nephridium of *Arenicola ecaudata*, × 20.

A. Grubii (Figs. 12 and 13) possesses the same type of funnel as *A. ecaudata*. It is flattened; the dorsal lip is semicircular, the ventral lip trilobate, with a small median lobe and large lateral ones, thus making this lip deeply notched. Ten or twelve blunt, digitate, ciliated processes attach to the dorsal lip; these are often branched and are provided with the blood sinus. The funnel attaches to the body at some distance from the anterior end and its axis is at right angles to the axis of the body. The bladder of the nephridium of this species is usually expanded: it is capable of equally wide expansion in *A. ecaudata* but is more often found contracted. In the other species, the bladder is not so distensible although it is relatively large at times, especially when filled with eggs or sperm about to be discharged through the nephridiopore.

I have collected *A. cristata* and *A. marina* near Woods Hole, Mass., *A. marina, A. Grubii* and *A. ecaudata* in the bay at Plymouth, England, and have studied fresh *A. Claparedii* and *A. Grubii* at Naples. I wish to express my sincere thanks to the directors of the biological stations at these several places, who have kindly placed at my disposal, material and facilities for the work, and to the Carnegie Institution whose table at Naples it was my pleasure to occupy.



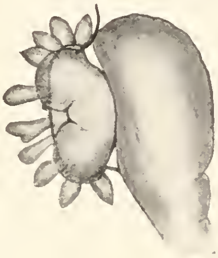


FIG. 12. The nephridium of *Arenicola Grubii*, × 15, dorsal view. The outline of the ventral lip of the funnel is shown in dotted line.

FIG. 13. Ventral view of anterior portion of the third left nephridium of *A. Grubii*, × 20.

BIBLIOGRAPHY.

Benham, W. B.

'93 Postlarval Stages of Arenicola marina. Jour. Mar. Biol. Ass. Plymouth, N. S., Vol. III., pp. 48–52.

Eisig, Hugo.

'98 Zur Entwickelungsgeschichte der Capitelliden. Mitth. Z. Stat. Neapel, Vol. XIII., pp. 1–293.

Fraipont, J.

'87 Le Genre Polygordius. Fauna u. Flora d. Golfes von Neapel, Vol. XIV., Monographie.

Freudweiler, Hedwig.

'05 Studien über das Gefäszsystem niederer Oligochaeten. Jen. Zeitschr. f. Naturwiss., Vol. XL., pp. 1–28.

Gamble, F. W., and Ashworth, J. H.

'98 Habits and Structure of Arenicola marina. Quar. Jour. Micr. Sci., Vol. XLI., pp. 1–42.

'00 The Anatomy and Classification of the Arenicolidæ. Quar. Jour. Micr. Sci., Vol. XLIII., pp. 419–569.

Lillie, Ralph.

'06 Structure and Development of the Nephridia of Arenicola cristata, Stimpson. Mitth. Z. Stat. Neapel., Vol. XVII., pp. 348–405.

Meyer, Ed.

'01 Studien über den Körperbau der Anneliden, III. Mitth. Z. Stat. Neapel., Vol. XIV., pp. 247–585.

Schiller, Ignaz.

'07 Ueber den feineren Bau der Blutgefässe bei den Arenicoliden. Doctor's Thesis, Univ. Zurich, Jena.

Willem, Victor.

'99 L'Excretion chez l'Arenicola. Travaux de la Station Zoologique de Minereux, Vol. VII.

Wilson, E. B.

'89 The Embryology of the Earthworm. Jour. of Morph., Vol. III., pp. 388–462.

Vejdovsky, Franz.

'05 Zur Hämocōltheorie. Zeit. f. wiss. Zool., Vol. LXXXII., pp. 80–170.

SOME OBSERVATIONS ON THE HABITS OF PECTEN DISLOCATUS.

B. H. GRAVE.

With the purpose of studying the habits of the scallop, *Pecten dislocatus*, I collected many young specimens ranging from two to ten millimeters in length and placed them in small glass aquaria in the laboratory.[1] They were found in the harbor at Beaufort, N. C., well above the muddy bottom, clinging to eel grass. They were usually attached by several strands of hyaline byssal threads, which were exceedingly strong and elastic.

Although the *Pecten* is so generally known as to make a detailed description of its anatomy superfluous, yet a brief description of certain parts is deemed necessary. For a more detailed study of the anatomy, reference can be made to a paper, by G. A. Drew, on "The Habits and Anatomy of the Giant Scallop."

By reference to Figs. 1 and 2, it may be seen that the shell is rounded and eared. The ears make possible the long, straight hinge line; which extends along their upper borders to their extremities. The right valve is slightly more convex than the left, and near the anterior[2] ear, it has a deep notch. This one feature mars the symmetry of the valves. Between the valves and just beneath the hinge ligament, there is a pad of cartilage-like substance, which is compressed when the valves are closed. It serves to open them quickly when the adductor relaxes (Fig. 3).

The form of the shell and the structure and arrangement of the soft parts within, adapt *Pecten* to the swimming habit. It swims by opening and closing the valves in rapid succession. By varying the position of the mantle so as to control the direction of the currents of water expelled from the mantle chamber, it is enabled to swim either forwards or backwards, although it usually swims with the opening of the valves directed forwards.

---

[1] Through the courtesy of Hon. Geo. M. Bowers, U. S. Commissioner of Fish and Fisheries, I had the privilege of occupying a table in the Fisheries Laboratory, at Beaufort, N. C., for two months during the summer of 1908. For this privilege and for many kindnesses shown me by the Director, Henry D. Aller, I am glad to express appreciation.

[2] The hinge line is here considered dorsal for convenience in description, although it does not represent the true dorsal of the animal.

A perfectly symmetrical shell is the form best adapted to swimming, and the presence of any irregularity in it, such as that just mentioned in the pecten, is to be explained either as an adaptation to habits other than swimming, or as a structure, inherited from an ancestral form, and not as yet obliterated through adaptation to the swimming habit.

Although adult specimens were kept in aquaria all summer, no method of locomotion other than smimming was noted, and no clue was gained as to habits which would in any way explain the function of the notch in the right valve. They neither attached themselves by a byssus, nor used the foot for locomotion. Young specimens, however, showed much more activity than the adults, and some observations on their habits are recorded in the following pages.

Concerning the function of the notch in a related species, *Pecten tenuicostatus*, Dr. Drew writes as follows: "I have been unable to satisfy myself as to the function performed by this notch. The sense tentacles on the mantle margin, opposite the notch, are somewhat longer than those adjacent, but I have been unable to determine that they have a special function or that they are especially advantageously placed."[1]

The Sense of Position.

The *Pecten* lies habitually upon the right valve and if placed upon the left, immediately turns over. When lying upon the left valve, it seems to feel the same sort of discomfort which a frog, or other animal with well developed balancing organs, feels when placed upon its back. However, after turning them over repeatedly, they sometimes remained resting on the left valve for several minutes.

The Asymmetry of the Valves.

When dropped through a considerable depth of water, *Pectens* settle about as frequently upon the left valve as upon the right.

---

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 7.

The slight flatness of the left valve does not serve to make them settle always upon the same side.

The Functions of the Foot.

The foot lies just opposite the notch in the right valve. It appears to be functionless in adult specimens, or rarely used by them, but is made use of to good advantage by the young. Specimens were often seen to extend the foot anteriorly to a remarkable distance, attach it at the tip to the bottom and then, by a powerful contraction, draw the body forwards to the point of attachment. The foot is cylindrical and seems very small to carry such a load; and frequently, after it has been extended and attached, the valves are opened and clapped together, at the same time the foot contracts, the body thus being drawn forward with much less strain upon that organ. This method of locomotion is a combination of swimming and creeping. The force of the current of water expelled from the mantle chamber serves to raise the body and propel it forwards as far as the attached foot will permit. At the same time, the foot contracts and the body lands close to the point of attachment. When this method of locomotion is used, the foot, instead of being extended directly outwards, anteriorly, is usually directed more ventrally, so that the point of attachment is more nearly in line with the force exerted by the swimming movement. Except for the notch in the right valve, this sort of performance would not be possible, because by the closure of the valves, the foot would be crushed.

The foot is, also, frequently used in turning the body over, when placed upon the left valve; it is extended anteriorly from the body and attached; the valves are opened and clapped together vigorously; the body, as a result, is raised and shot forwards, but the weight of the foot and the resisting pull from its attachment cause it to swing over upon the foot as a pivot, the scallop landing upon the other valve, having turned through an arc of 180 degrees.

The above method of turning over, is usually, if not quite universally, used by specimens when placed upon the left side for the first time. After a little handling, however, they become much more irritable, seeming to be excited, and at such times, they manage to right themselves by one of three methods: Sometimes without extending the foot, they open the valves and clap them together. After one or several trials, the body turns over upon the hinge line as a pivot. The mantle must have played a part in this by expelling currents of water in a direction such as to cause the body to turn over.

At other times, a position on the right valve is gained by one or more short swims, the method being continued until the body comes to rest on the right side. They usually manage to alight upon the right valve after a few trials, and then they become quiet.

The Byssus.

When specimens are allowed to lie undisturbed upon the right valve, they usually become attached by numerous strands of strong byssal threads. A short time only is required for this to take place. They frequently become firmly fixed in from two to five minutes and the threads are sufficiently strong to support a weight several times that of the body of the *Pecten*. The byssal threads pass through the notch in the right valve directly to the support below. They adhere, to some extent, to the shell where they come into contact with it.

So long as specimens are kept lying upon the left valve, they cannot, or do not, attach themselves by the byssus. Since the byssal gland lies at the base of the foot, it is possible that the notch, in the shell opposite it, is a structural adaptation directly correlated with the function of the byssus. At any rate, because the byssal threads extend through the notch, in place of over the edge of the shell, the pull has less tendency to tilt the body than would be the case if no notch were present.

In order for the byssus to become attached to the bottom, it is not necessary for the valves of the shell to be opened, since the attachment of the byssus is frequently accomplished while they are closed. The notch in the shell is sufficiently large to allow the extension of the foot to the support during the process of attaching the byssus. It seems that Dr. Drew has observed this process in individuals of *Pecten irradians*, to quote:

"An individual of *Pecten irradians* placed in a glass dish of sea water will sometimes protrude its foot from the shell, apply it closely to the bottom of the dish and after a short time, slowly withdraw it, leaving a rather broad band of slightly yellowish material attached to the glass and connected with the foot by the byssal gland. This is not composed of small threads as in the mussels *mytilus* and *modiola*, but it may be sufficiently tough to support the weight of the animal, if, after a few minutes, the dish is carefully turned over."[1]

SUMMARY.

By way of summary, therefore, it might be said concerning the function of the notch that it makes possible a much freer use of the foot and byssal gland, and is in some way connected with the function of these organs. Although many mollusks live in the mud, the fact that young *Pectens* do not is evidence that they do better out of it. The foot and byssus enable them to climb upon supports and maintain their position there. As they approach maturity, they assume more and more the swimming habit and the foot and byssus lose, to some extent or entirely, their functional activity. If these organs are not functional in full-grown *Pectens*, as seem probable, the notch is no longer of any value to them, although it is not obliterated.

The *Pecten* has the sense of position well developed.

EARLHAM COLLEGE, RICHMOND, INDIANA,
March 6, 1909.

---

[1] *The University of Maine Studies*, No. 6, September, 1906, p. 18.

EXPLANATION OF PLATE I.

FIG. 1. Shows the left valve as it appears from surface view. It is not quite symmetrical.

FIG. 2. Shows the right valve as it appears from surface view. The prominent notch at the base of the anterior ear can be seen.

FIG. 3. Is a view of the inner surface of the right valve. The dotted line shows the position of the cartilage pad which aids in opening the valves.

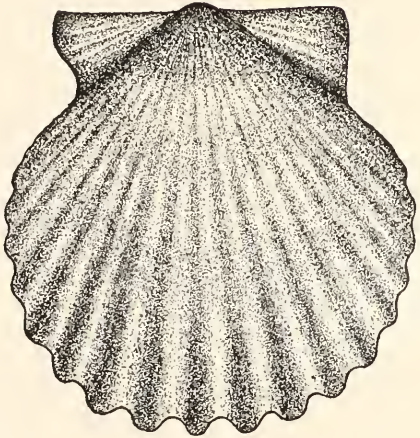


Fig. 1

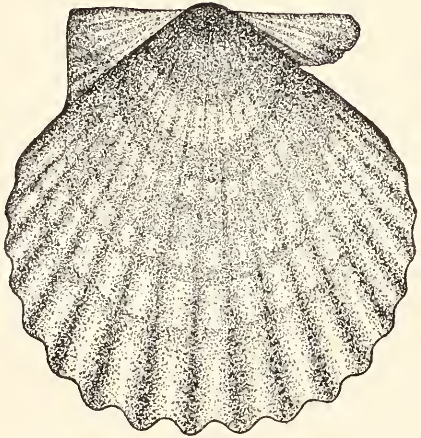


Fig. 2

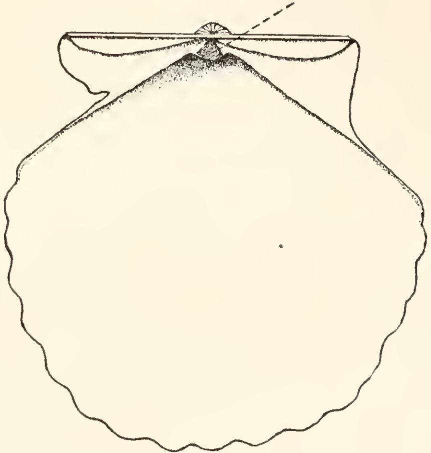


Fig. 3

THE DYNAMIC FACTOR IN REGENERATION.

T. H. MORGAN.

With the publication of the data here presented the series of experiments that I have carried out on *Tubularia* for several years may be considered as temporarily brought to a close. I take this opportunity therefore to sum up the evidence bearing on the problem of the formative factors of regeneration, as exhibited by this hydroid. In the course of my experiments tentative hypotheses have been proposed here and there that have at least served to suggest further experiments. The conflicting evidence sometimes inclined me towards one point of view, sometimes towards another; yet, all in all, the same general line of thought, if sometimes vague, can be traced through the attempts to analyze the results. It will be my endeavor here to bring more into the foreground those theoretical deductions that seem to me at present to be best in harmony with the experimental evidence.

That the dynamic factor in regeneration is not primarily the outcome of physiological movements of the animal, or of its parts, is made probable not only by many facts familiar to every student of regeneration, facts that show that the new part often develops under conditions where movement or function in this sense is absent, but also by the important experiments of Zeleny and of Stockard with the jelly-fish *Cassiope*. They have shown that when one half of the disc is brought to rest by removing the sense organs (and scratching a barrier zone across the connecting ectoderm) the quiescent half regenerates as well and as rapidly as the half left pulsating during the period of regeneration.

Since every part of the stem of *Tubularia* is capable of producing a hydranth the inhibition of basal development is obviously due to the presence of a hydranth or of a developing hydranth at the oral end. Two alternative chemico-materialistic explanations have been suggested for cases like this. (*A*) The oral hydranth may use up some materials necessary for the formation of a basal hydranth. (*B*) The hydranth may produce some materials that inhibit the development of other hydranths from the remainder of the piece; hence the inhibition, as long as a hydranth is present or developing. On first thought these alternatives would seem to cover the only possible ways in which the problem of regeneration may be presented — at least as long as the problem is confined to purely physiological actions of a chemical order. There are, however, not a few considerations indicating that the fundamental interpretation may lie in a different conception of the problem. I shall try here to emphasize this other point of view without attempting to develop it into a theory of regeneration. At most we may hope at the present time to find in the facts some indication of the nature of the problem if not its entire elucidation.

A number of experiments have been made that seem to indicate that the temporary inhibition of the development of the basal hydranth in *Tubularia* is not the result either of the using up of materials by the oral hydranth, or of the setting free of inhibitory stuff. The simultaneous development of hydranths at both ends of a piece, which frequently occurs in short pieces, is a case in point. Both ends develop at the same rate as when a single hydranth develops, and not half as fast as the hypothesis demands. Again the development of a basal hydranth does not appear to inhibit the oral development as we should expect if the result were dependent simply on the presence of materials in the stem. Some experiments of MacCallum's with plants have an important bearing on this point. If the terminal bud of the bean is removed, the buds in the axils of the cotyledons develop. But if the activity of the terminal bud is simply lessened by inclosing that part in an atmosphere of hydrogen, the basal buds do not develop. Hence the result is due not to activity of the terminal end, but to its presence or absence. In a different way the same fact is brought out. A piece of willow stem is cut off, its middle third is inclosed in a tube filled with moist air, so that the buds in this part are encouraged to begin their development; the dry air retarding the development of those outside. After the middle buds have unfolded, the entire piece is inclosed in a moist chamber, when the more apical buds sprout forth, while none of the buds basal to the middle region develop. The presence of growing shoots in the middle of the piece does not inhibit the apical buds from developing, if external conditions are supplied favorable to their growth, but the basal buds are inhibited by the presence of shoots on the more distal parts. These facts are incompatible with the assumption that the results are due to the presence of materials used up by those parts that develop first to the exclusion of other parts. They also show that the alternative view is untenable, for, the presence of growing shoots in the middle of the piece is not antagonistic to the development of shoots in other regions provided those regions are situated more distally.

In the case of *Tubularia*, it is more difficult to present convincing evidence that distal hydranths do not produce materials inhibiting the development of basal hydranths, improbable as such an interpretation may now seem. But the fact that basal hydranths do develop after the oral hydranths have formed may seem to discredit this view. Here, however, an apparent paradox is found. The experiments seem to show that when the oral hydranths develop, the basal hydranths are retarded in development, but they do develop later, and the results also show that if both start simultaneously both develop at the normal rate. The paradox is due, I think, to two antagonistic factors at work at the same time. Admitting that the oral development tends to inhibit the beginning of basal development, we also find that if other influences suffice to start both simultaneously, the on-rush, so to speak, of the process once begun changes the conditions that tended to prevent the starting. Strange as this seems it is little more than a statement of the facts. The same results may be put in a somewhat different way. A cut end being present, whether oral or basal — the conditions that call forth hydranth formation are given. Experiments show that the oral end tends to develop first, its development acts as a partial inhibition of the basal hydranth-formation. If this influence is strong enough the basal development is temporarily held in abeyance, but if not the inhibition is overcome. Once overcome, the formative influences do not check the further action of the basal end. In this connection it is curious to note that small oral pieces produce simultaneous hydranths more often than larger pieces. The interpretation of this seems to be that the tendency to produce hydranths, both oral and basal, is stronger near the distal end and decreases basally. In short pieces the sensitiveness of the two ends to those influences that call forth the hydranth is so great that both ends develop simultaneously or nearly so, hence the oral end has not time to get a sufficient start over the basal to stop its development. It should be noted in passing that it is probable that the influence preventing basal development is not only the oral development, but a direction-factor present in the stem at all times. This factor we call polarity. The interesting point is that this factor seems to be more capable of inhibiting basal hydranth formation when an oral end is developing than when such development has not yet begun. The basal development, however, does not appear to delay the oral process. It is acting against the polarization and its influence is less felt throughout the stem, as experiments by Stevens and myself have shown. These considerations lead, I think, to the view that the essence of our problem lies in that peculiarity of the piece that we designate its polarity, and not in the absence or presence of formative substances in Sach's sense.

If our analysis is correct, we are led to look upon living material as possessed of a certain formative principle that has so to speak a "sense of direction." The next step will be to study the nature of this principle and see what properties we are justified in ascribing to it; for while it may be beyond our powers at present to state precisely the nature of the directive principle, we may at least be enabled to work out its manifestations. Some of these manifestations become apparent in the study of the regeneration of *Tubularia*. One of its most striking modes of action is seen in the inhibition of basal-hydranth formation. Most interesting is the result that its action becomes intensified by developmental processes going on at the oral end, as shown by the fact that if the oral development is suppressed by tying that end, the basal development is much accelerated. It is accelerated in the sense that basal hydranths more often develop at once than when both ends are open, but not in the sense that the basal development is faster than when this end also gets as early a start as the oral end. In other words, there is no speeding up of hydranth formation as such, but the initial inhibition is overcome.

The special problem with which this paper deals is the nature of what takes place at the basal ends when the oral end is kept open and when it is tied. Is the retardation of such a kind that a slower process of development is going on at the basal end while the oral end is developing, or does the basal end not really begin to develop until the oral end has formed its polyps. If so, what gives it its start later? The following experiments were devised to study these questions.

Experiment I. — The purpose of the experiment was to determine whether when both oral and basal ends of a piece are left open constructive changes are slowly going on at the basal end. Some pieces were cut off and left open (*A*); later other pieces were cut off and the oral ends tied (*B*) and at the same time the oral ends of (*A*) were tied. It was found that the basal ends of the (*A*) pieces did not develop faster than those of the (*B*) pieces, showing that the changes at the basal end of (*A*) are not progressing, but are held in check by the developing oral hydranth.

Control I. — In some pieces the old hydranths were left intact and the pieces cut off. No basal hydranths began to develop until the old heads began to be absorbed. The presence of the old heads inhibited the development of the basal hydranths until the heads had degenerated when the latter appeared.

Experiment II. — In order to find out whether, when the oral end is tied, changes take place throughout the piece that tend to make more rapid the development of basal hydranths, or whether these changes are localized at the basal end where the new hydranth develops, the following experiment was tried. The oral ends of many pieces of the same length were tied. Then after several hours' interval differing in several experiments, the basal end was cut off, (*a*) just inside of the area that would form the basal hydranth, (*b*) in the middle of the piece, (*c*) just below the ligature. In general the development of the basal hydranth was delayed as compared with control pieces tied but not cut off at the basal end; the delay was the greater the further removed the cut from the basal end, despite the fact that oral levels tend to regenerate faster than more basal levels. The differences are more apparent the longer the time that elapses before the basal pieces are removed. The differences are not very marked at the different levels indicating perhaps that changes take place throughout the piece and not only at the basal end although more pronounced in the latter. The different levels of the cuts make it difficult to ascribe the results solely to the general changes in the piece, for the more orally situated cut ends have an advantage in level as other experiments have shown.

Experiment III. — Pieces were cut off at the same oral levels. After 23 hours the hydranth region at the oral end was cut off of some pieces (*A*), others were cut in two in the middle of the piece (*B*), and for a control some pieces were left as before (*C*). A slight retardation occurred after another 12 hours in (*A*), less in (*B*) as compared with (*C*). Removal of the hydranth forming region after 23 hours causes delay but the delay is not so much as though a new hydranth had developed at the new cut, showing that changes directed towards hydranth formation are going on not only in the region where the hydranth will develop but at more basal levels as well.

Experiment IV. — This experiment was like the last, except that the basal ends of all the pieces were tied, thus preventing the basal end from exerting any influence on the result. Other experiments had shown, however, that the basal development, even if it occurs, has apparently no retarding influence on the oral development. The results, as was to be expected, were the same as in the last experiment. It is interesting to note that in both the influence of the cutting causes a greater delay in the first appearance of the primordia of the hydranth than on their later development; for later the differences seem to be less than at first. This may be due to an acceleration extending throughout the whole time that is more effective after a beginning has been made than before the start.

Experiment V. — Some previous experiments had left undecided the question whether, when the oral end is left open for several hours and is then tied off, the basal development is more rapid than when the oral end is tied at once. If such an acceleration really occurs it might seem to indicate that changes take place in the oral end that produce accelerating materials even for the basal ends. I was particularly anxious to settle this point definitely, for obviously, if such acceleration could be proved, it would furnish evidence in favor of a chemical process, especially since other experiments had seemed to show that the basal end does not begin its development when both ends are left open. I have carried out rather an extensive series of experiments that give, I think, a definite answer to the question.

When pieces are left open at both ends from four to nine hours, and are then tied at the oral end, the basal development is slightly retarded as compared with its development in pieces tied at once. There is little evidence in favor of the view that the later tied pieces can make good the loss of four to nine hours, and of course they can not catch up if a longer time elapses. Whether they may do so in later stages is more difficult to decide, but this does not concern the main point here raised.

Individual differences in rate, differences in stems, and uncontrollable differences in level tend to obscure results that depend on only four, six, and nine hours differences in start. The above statement holds, therefore, only for average results. There was found no evidence in favor of actual acceleration, whether there is some relative acceleration is difficult to decide. If the hydranths do not develop promptly *i. e.*, if a long time elapses between the tying and the appearance of hydranths, the initial differences of a few hours may be lost.

Experiment VI. — Another attempt was made to see whether changes take place in the piece as a whole, after it is cut off, that make more rapid the development of an *oral* hydranth when a new cut is made.

Pieces were removed and after four hours somewhat more than the oral hydranth region was cut off. In some cases the newly cut ends developed as fast as did the hydranth in the small pieces cut off, but the latter may have been retarded by the operation or by the smallness of the pieces ; yet in some cases the development of the newly cut ends was as rapid as in control uncut pieces. This result indicates that changes take place in the pieces behind the actual region of hydranth formation that lead toward the development of a hydranth.

Experiment VII. — In this case pieces first cut off were after ten hours cut in two in the middle. Comparing the rates of development of the oral ends of the oral halves with that of the oral ends of the basal halves it appears that the latter are slower, but there is evidence that the retardation may be somewhat less than the ten hours difference in initial start. The basal halves in this experiment are also somewhat behind the basal halves in the last experiment, which seems to show that the general changes in the excised pieces that go towards oral development decrease from the cut ends inwards.

It has not seemed necessary to give the details on which these general conclusions are based. The nature of the case makes it difficult to obtain results as definite as one might wish, despite the precautions that were undertaken to make the conditions as uniform as possible. The general conclusion that changes take place in the piece as a whole, after its removal, that are in the direction of hydranth formation, seems fairly certain. Less certain perhaps is the evidence to show that when the oral end is tied similar changes take place in the pieces that accelerate basal development in regions beyond the hydranth forming region, but this conclusion too is, I think, quite probable. The nature of these changes is not revealed.

The Dynamic Factor in Egg-Development.

Students of the processes of regeneration have without exception made use of the term polarity to express a directive factor observable in their results, and to this factor is sometimes ascribed an active rôle as a controlling influence, at other times the term is used descriptively merely as a statement that the new structures are directed in the same way as the part removed. In both respects the word has been useful, however vague our conception of what polarity may be. Our analysis of the process has now gone sufficiently far, I think, to justify us in an attempt to come to closer quarters with the term. Without reviewing the opinions that have been expressed as to the nature of polarity, I shall try to contrast two views of its nature that seem to me to represent the two main lines that speculative thought has followed. It should be noted that the term is used equally by students of embryology and by students of regeneration. The former finds axial relations in the egg — polarity, bilaterality, radial symmetry, etc. ; the latter finds the new organs regenerated in definite relations to the old. To some observers the distribution or the stratification of the materials of the egg, has seemed a sufficient basis for the results referred to under the term polarity ; to others it has seemed more probable that there exists in the egg an arrangement or structure that has axial relations from which result not only the depositions of the formed materials but also the nature of the action of the parts. Polarity is from this latter point of view not simply a passive structure, but a relation of the parts that directs the shifting series of changes that we call development. At one time one of these views has seemed more probable ; at other times the other. The history of modern experimental embryology and regeneration shows the influence that these views have had on those who have followed the new work. In a general way the two views may be classed as the materialistic or chemical and the dynamic or physical conceptions of the developmental process. At present it seems to the writer that the evidence has been steadily pointing to the second of these contrasting views as the more probable. As far as the egg is concerned, the recent experimental work goes to show that the visible inclusions of the protoplasm (yolk, oil and other granules perhaps) are not the fundamental causes of the formative processes, although they may be needed in certain regions to carry out the future development of the structures that there appear. In regard to regeneration it has been evident for some time, that the specification or the differentiation (with its concomitant products), cannot be unreservedly utilized as a basis for an explanation of formative processes that take place. For example, if the gross materials or the differentiations of the head end of a planarian are the causes of that region being a head, it is inexplicable that when the head is removed it could regenerate a tail. There must be something else behind what we see that is responsible for the change that takes place. These and other considerations lead to the view that there exists a fundamental property of living matter that is the formative principle of development. On two former occasions, when attempting to analyze the results of regeneration in *Tubularia*, the author tried to account for the results of polarity on the basis of a stratification of the materials. Influenced at the time by recent results in experimental embryology that seemed to show that visible substances of different kinds in the egg are really responsible for the development of its parts, the same idea was applied to the problem of regeneration, despite the fact that I had on more than one occasion rejected the hypothesis of formative stuffs, in Sach's sense, as sufficient to account for the facts of regeneration. Yet a careful reading of the papers here referred to will show that I still held, though perhaps not always consistently, to the conception that back of these differentiated materials lay the real differentiating factors.[1] It now seems to me that the evidence, which at that time seemed so strongly to favor the idea of the importance of the grosser materials of the egg, is insufficient to establish its case, and that the important factors of development are dynamic properties of the bioplasm, rather than the formed products of the egg, or of the differentiated products of the adult animal. This statement does not mean that the visible products in the egg play no rôle in development. The evidence still shows that they may do so, but their rôle seems to be secondary, not primary.

The interrelation of the parts seems to be one of the most evident expressions of the fundamental formative influences. Several years ago a consideration of a number of results in regeneration led me to state that this relation might be expressed as a sort of tension. This view has been objected to on the ground that it does not appear to explain the matter any better than before. In a moment of doubt and in order to give the statement a meaning for those who believe no suggestion to be of value unless it refer the problem to ordinary properties of inorganic bodies, I suggested that osmotic pressure might be the cause of the tension differences in the parts. This was an unnecessary concession. The behavior of fluid crystals (according to Lehmann) shows that the formative changes can be accounted for on the basis of a tension exhibited by the molecules of the substance of the crystal. While the organism may not be put down as a fluid crystal, still we see that physical properties other than osmotic pressure and surface tension may play an all-important rôle in form-changes. It may be that a similar property is the cause of the formative changes in the organism. In any case, the facts that I had in mind suggested that tension of some sort is an important dynamic factor in development, perhaps *the* important factor. The facts still seem to me to indicate some such relation between the parts, and no one regrets more than I that we cannot "explain" the results even if my suggestion prove to be in the right direction.

---

[1] One further word of explanation. The rate of hydranth formation varies with the distance of the cut end from the original hydranth. I have spoken of this difference in rate as explicable on the assumption of the hydranth-forming materials decreasing toward the base, *i. e.*, away from the hydranth. It was unfortunate to have used the term hydranth materials, although I made sufficiently clear in the text that I did not mean to invoke the stuff-hypothesis in this connection. It is not entirely clear on what the difference in rate depends; most probably on the stem being less specialized as a store-house of food substance nearer the hydranth; probably also on some difference connected with the thickness of the walls with which the specialization may also be connected; possibly neither of these but some more fundamental characteristic is responsible for differences in rate. In any case it is not obvious that there is any connection between this difference in rate and the polarity of the piece. The latter is the same for all levels — the time it takes the piece to be remodelled seems to be referable to something else.

Still later a consideration of certain facts of development led to the suggestion that two known properties of the organism — contractility and irritability — also play a very important rôle in embryonic and regenerative development. I shall not attempt to review here the argument which led to this point of view. How far and in what sense contractility and irritability are better expressions of the tension hypothesis, it is not easy to state. So far as contractility is concerned Lehmann's recent important paper on "Scheinbarlebende Kristalle"[1] shows the possibility at least of referring this property also to a condition of molecular tension. We are still too ignorant of the physical basis of irritability to make speculations in this direction profitable, but it may be well not to lose sight of this property of living matter in our attempts to analyze further the problem of development.

STEREOMETRY OF THE BIOPLASM.

Polarity implies difference in one direction. Every student of regeneration knows that in all three dimensions of space the same factor is present. Polarity is therefore only a part of the problem, and so far as it draws attention away from the whole problem it seems best to substitute the term stereometry.

---

[1] *Biol. Centralb.*, XXVIII., 1908.

Sufficient evidence has accumulated, I think, to show that stereometry has a dynamic side — in so far as it is a result of the molecular factors that determine the relations of the parts to each other. A question of fundamental importance here presents itself. If the formed substances at each level are the products of the bioplasm, must not the bioplasm itself be stratified in nearly the same sense? It was this idea that I had in mind when I wrote in 1906: "If we imagine a stereometric network as a part of the specialized structure, we must be prepared to admit that it changes at each level as the structure changes. Therefore it seems to me simpler to base our hypothesis of polarity on the difference in differentiation itself, and not on an imaginary polarized system associated with the living materials." But the point I overlooked was that there is no need to suppose that a heterogeneous network of bioplasm exists because the visible structure formed by it is different. The relation of the polarized material to the ends of the material (indeed to all its directions) suffices to account for the difference of level. In fact if the stereometry rests on a dynamic and not a statical relation of the parts this is the logical standpoint.

It has been suggested that irritability may be related to the dynamic factor of development.

The effects of irritability at any level may be realized through the chemical changes inaugurated. These chemical changes once started may, if enzymatic, thenceforward continue (*unless checked by other chemical processes*), *independently of the factor that set them going.*

Vol. XVI *May, 1909.* *No. 6*

BIOLOGICAL BULLETIN

THE REGULATORY CHANGE OF SHAPE IN PLANARIA DOROTOCEPHALA.

C. M. CHILD.

The recent discovery of *Planaria dorotocephala* Woodworth (Woodworth, '97) in very large numbers, near Chicago, has made it possible for me to use this form for extensive series of experiments. The species is very similar to *P. maculata* in structure, behavior and regulation, but possesses some advantages over that species for experimental work. It attains a larger size, is more active, and can be obtained in unlimited numbers and all ages in this locality, while *P. maculata* is much less abundant. I found the same species in California some years ago (Child, '06), but was unaware at that time that it had been described.

In the present paper only certain experiments concerning the effect of anæsthetics on form regulation will be considered. It is possible by the use of dilute solutions of anæsthetics to control, modify and inhibit various regulatory processes almost at pleasure. For example, head-formation can be made a process of redifferentiation instead of regeneration in almost any desired degree (Figs. 14 and 16) or can be completely inhibited, according to the conditions of the experiments, and the same is true concerning the formation of a new posterior end and a new pharynx, and the regulatory changes in the intestinal branches. Moreover, the use of anæsthetics permits, in greater or less degree, an analysis of some of the various factors concerned, and finally, it is possible by this means to produce individuals capable of continued existence if returned to water which possess characteristics, or perhaps more properly, combinations of characteristics which do not occur in nature.

The anæsthetics chiefly employed in my experiments thus far are alcohol, ether and acetone-chloroform, commercially known as chloretone. The effects of all are essentially similar in kind, but of course differ quantitatively according to the substance and the concentration. Ether, for example, in a concentration of 0.4–0.5 per cent. produces about the same effect as alcohol in a concentration of 1.5–1.6 per cent. In solutions of these concentrations, individuals and pieces have been kept alive as long as three months, though the resistance differs greatly according to the condition of the animals and various other factors, most of which can be controlled experimentally either directly or indirectly.

In order to avoid as far as possible decrease in concentration of the solution by evaporation the following method has been used. The animals or pieces are placed in Stender dishes of several hundred c.c. capacity with ground edges and a cover with ground groove exactly fitting the edge. The groove is filled with solution of the same concentration as that in the dish so that the dish is sealed so long as the fluid does not evaporate from the groove. After the dishes are thus closed they are placed in larger jars containing a liter or more of the same solution and these are sealed with vaseline and the covers weighted so that no escape of vapor or entrance of air is possible. And finally, all solutions are renewed every forty-eight hours and are made up immediately before using. In this way it is possible to compare the effect of the anæsthetic upon pieces of different size and from different regions of the body and also upon worms in different physiological condition.

This method makes possible the control and modification of form regulation in this species to a greater extent than any other which has been described. At present, however, only certain points will be considered, a full account of the experiments being postponed to a future time.

In several of my "Studies on Regulation" I discussed the changes in shape and proportion which occur in isolated pieces of various species of turbellaria and which, under the usual conditions, constitute an approach to the shape and proportions of the whole animal. These experiments with anæsthetics furnish new data which confirm my earlier conclusions, and it is some of these data which are to be presented here.

I. Experimental Data.

When whole individuals or pieces are placed in 1.5 per cent. alcohol or in 0.4–0.5 per cent. ether they lose the power of coördinated movement almost entirely for a time. After four to five days, however, they become in some degree acclimated to the new conditions and begin to move about very slowly, but with increasing vigor as time goes on, though they never attain the normal motor activity. Pieces including the old head begin to move about earlier than pieces without a head, for the latter must form a new head before they can regain the usual degree of motor activity, and regulation is greatly delayed in the solution. The important point for the present purpose is that for some days movement, and particularly coördinated locomotion, is almost completely eliminated. It is of interest to determine to what extent regulation occurs under these conditions.

The first experiment to be described concerns pieces including that part of the body anterior to the line *b* in Fig. 1, *i. e.*, short pieces with the old heads. In Fig. 2 a piece of this kind after ten days in 1.5 per cent. alcohol is shown: during this time the piece has moved about but little and that chiefly during the last few days. Fig. 6 represents a similar piece after five days in water, Fig. 7 the same piece after ten days. Comparison of Figs. 2 and 7 shows that regulation in the alcohol is greatly delayed: a little new tissue has been formed at the posterior end, but, as a microscopic examination under pressure shows, it is still a mass of cells without any marked visible differentiation, and it can readily be seen that it is not used as a tail and is not attached to the substratum as the animal creeps slowly about; a small group of cells is present in the pharyngeal region, but these likewise show no marked differentiation. In water, on the other hand, a new tail has been formed which functions in the normal manner, contracting, extending and attaching to the substratum as the animal

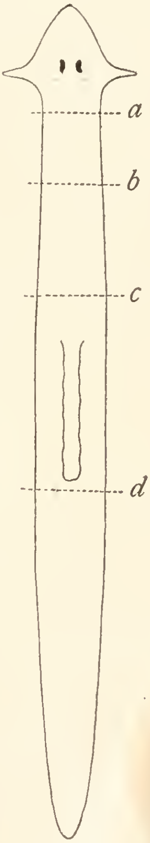
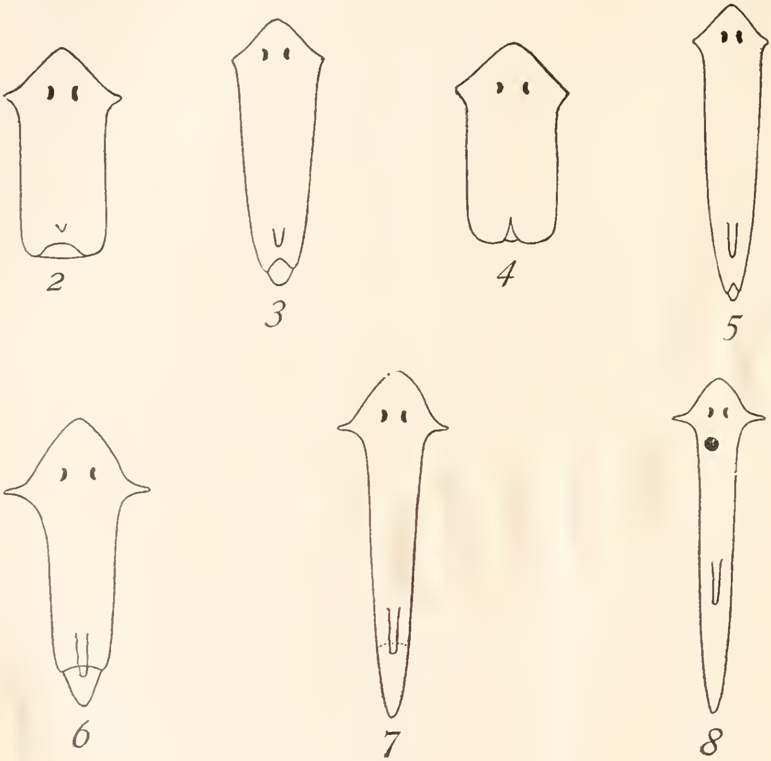


Fig. 1.

creeps; the pharynx is well-developed and sections show that it possesses essentially the same structure as the pharynx in uninjured animals.

But the difference between the two pieces is most marked as regards change of shape. The piece in alcohol has not elongated at all, in fact it has decreased in length and it may be noted incidentally that the "auricles" on the sides of the head are greatly reduced. The piece in water (Fig. 7) has in the same length of time elongated to nearly twice its original length, has become much more slender and tapers posteriorly. This piece has moved about during regulation to an even greater extent than the uninjured animal, for short pieces with the old heads are usually more active than whole individuals.



Figs. 2–8.

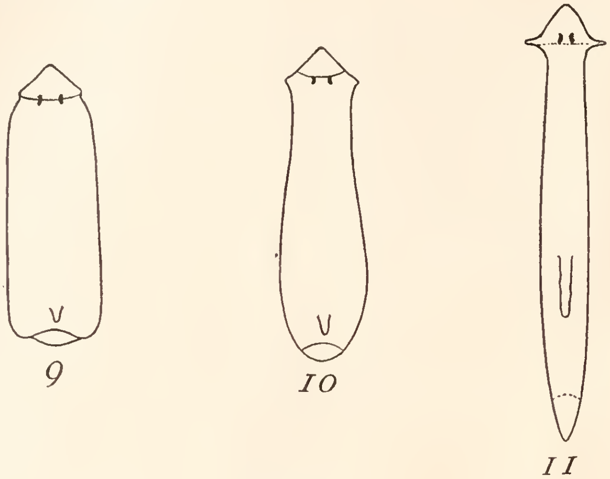
After ten days the piece in alcohol gradually becomes more active, though it never attains anything like normal activity. At the end of twenty or twenty-five days it has acquired a shape like Fig. 3. The posterior end now functions as a tail to some extent and attaches itself to the substratum as the animal advances but the amount of new tissue has not increased. The piece may live for six weeks or more in alcohol but it never undergoes any appreciable further change in shape. The newly formed parts undergo some degree of differentiation but never attain the characteristic adult structure. Apparently the piece has attained approximately a condition of equilibrium for the conditions under which it is living.

If the concentration of the solution is gradually increased after the first three or four days it is possible to inhibit practically all regulation beyond the closure of the wound: the pharynx does not appear, no further growth of new tissue at the posterior end occurs, and the piece undergoes no elongation (Fig. 4). Under these conditions the piece does not acquire the ability to move about.

If now these pieces which have attained equilibrium in alcohol be returned to water they gradually resume the process of regulation, but with certain differences from pieces which have not been in alcohol. The chief difference for present purposes is that the outgrowth of new tissue at the posterior end does not proceed until it reaches the usual amount. The tail is formed almost entirely by a redifferentiation of the old tissue (Fig. 5). The pieces may undergo change of shape after their return to water until they attain practically the same shape as pieces which have not been in alcohol. Fig. 5 shows a later stage of Fig. 4 after its return to water and Fig. 8 will serve as regards shape for the late stages of either water or alcohol-water pieces.

These results, which are merely illustrations of what I have observed in several hundred pieces, permit certain conclusions of interest. In the first place it is possible to inhibit entirely the change in shape without inhibiting entirely the processes of redifferentiation and regeneration, and the change in shape can be stopped at any point without stopping entirely other regulatory processes (Fig. 3). On the other hand, if the growth of new tissue from the cut surface is inhibited in earlier stages (Figs. 2

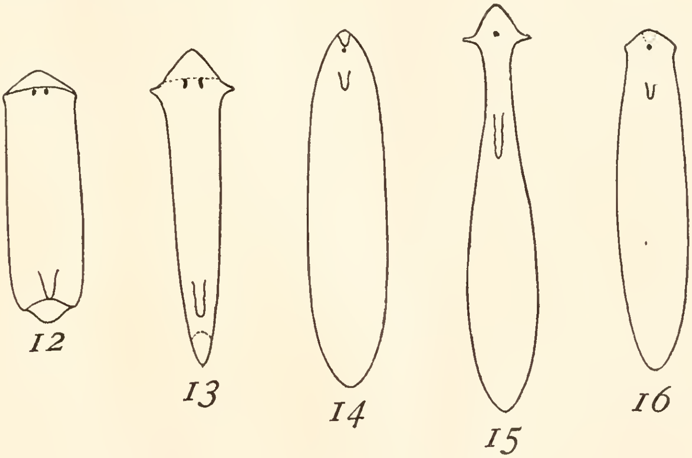
and 4) the change in shape may occur in later stages (especially after return to water) without the formation of more new tissue (Figs. 3 and 5). It follows that the factors determining the change in shape must be in greater or less degree different from those determining the localization and growth of new parts. Moreover, the change in shape occurs only when the piece is capable of locomotion and it is in general proportional to the locomotor ability of the piece.



Figs. 9–11.

But pieces which do not possess the old head afford even more positive evidence for these conclusions. Fig. 9 represents a piece corresponding to the region between the lines *a* and *c* in Fig. 1 after fifteen days in 1.5 per cent. alcohol. A new head has appeared, a small new pharynx is present as a mass of undifferentiated cells, and some new tissue has formed at the posterior end, but the piece as a whole shows no approach to the normal shape : it has undergone no marked changes in proportion. Incidentally it may be noted that the pharynx in such pieces appears much further posteriorly than in similar pieces in water. After seven days more the piece has the shape shown in Fig. 10. It moves about slowly, but its movements are different in character from those of pieces possessing the old head : here the posterior half of the body is very evidently not under complete control, *i. e.*, is not fully coördinated with the anterior region, and when the animal advances it is simply dragged along as a mass of inert material, its posterior end being only very rarely attached to the substratum. The shape of the piece suggests that the anterior part is being stretched by the strain upon it of the posterior portion, and I believe that is exactly what is occurring.

Similar pieces in water attain in the same length of time the shape and structure shown in Fig. 11. Pieces in alcohol of 1.5 per cent. do not change in shape much beyond the condition shown in Fig. 10, but if they are returned to water they regain their normal locomotor activity and may finally reach a shape like that of Fig. 11.



Figs. 12–16.

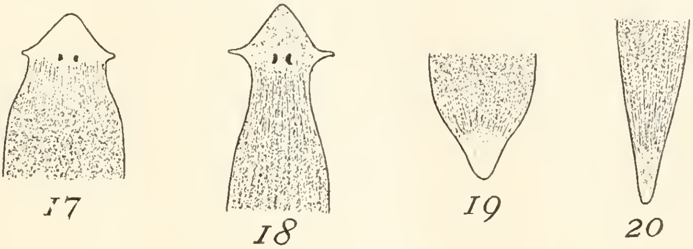
In these cases a new head, a small new pharynx and some new tissue at the posterior end have appeared without any marked change of shape in the piece as a whole (Fig. 9). Evidently the change of shape and the localized formation of new tissue are not necessarily correlated.

The same thing appears in Fig. 12, which shows a piece from the same region (*a* to *c*, Fig. 1) after fifteen days in 0.5 per cent. ether. Head, pharynx and posterior end have formed but no change in shape has occurred. This piece was returned to water at this stage and after seven days more had attained the shape shown in Fig. 13.

In another series pieces comprising the whole post-pharyngeal region were used (posterior to *d*, Fig. 1). These pieces, after eighteen days in 0.5 per cent. ether, had attained the condition shown in Fig. 14. A small head is forming, almost entirely by redifferentiation, at the anterior end, with one median eye, and a small pharynx is present. At this time half of the pieces were returned to water and half remained in ether. After nine days more the pieces in water had acquired the condition shown in Fig. 15 while those in ether were like Fig. 16. In the pieces returned to water the anterior half is greatly elongated but the posterior half remains much as before. In these pieces, as in the one described above, the posterior part was dragged about by the more active anterior portion. Gradually complete coördination returned and the posterior end began to attach itself to the substratum as the animal advanced and after this the shape gradually approached that of the normal animal.

Further data along this line could be presented but these cases are sufficient to show that it is possible to delay or inhibit the change in shape to any desired degree, and to induce its occurrence at any time. Moreover, the manner in which it occurs can be controlled and altered indirectly by using pieces of different sizes and from different regions of the body.

To my mind the evidence indicates very clearly that the change in shape is primarily a mechanical deformation of the body in consequence of the altered direction of the strains to which it is subjected as the animal advances. To control the change experimentally we have only to control the locomotor activity. In several of my earlier papers (Child, '02, '03, '04*a*) I have described the method of locomotion in certain species of turbellaria: in *Planaria* longitudinal strain arises in essentially the same manner as in the other forms discussed, the use of the posterior region as an organ of attachment being one of the chief factors. Moreover, there is considerable direct evidence that the tissues of a region undergoing change of shape are being stretched. In regions where the decrease in width and the elongation of the old parts begin, the chromatophores are always greatly elongated, and their elongation is greatest where the change in shape is most rapid. As the change goes on they become drawn out into long lines. Figs. 17 and 18 indicate this change in shape of the chromatophores in the region posterior to a new head, and Figs. 19 and 20 for a region anterior to a new tail. In the pieces in alcohol and ether this change in shape of the chromatophores appears only when the change in shape occurs, not when the new tissue is formed. In cases where the change of shape is inhibited in the anæsthetic (Figs. 2, 4, 9, 12) it does not appear at all, but if such pieces are returned to water, and the change of shape occurs, the stretching of the chromatophores also appears. In Fig. 14, for example, it did not appear so long as the piece was kept in the ether, but after several days in water it was most conspicuous in the slender anterior region (Fig. 15), this region appearing almost as if finely striped in the longitudinal direction. This change in shape of the chromatophores is actually a stretching, not a migration, for it is possible to select some particular spot which happens to be conspicuous for some reason and to observe its change of shape from day to day: in such cases it can be seen clearly that merely elongation not migration occurs.



Figs. 17–20.

A similar elongation is visible in the parenchyme cells in section. Stevens ('07) has recently described this elongation or orientation of the parenchyme cells and regards it as indicating migration, but Steinmann ('08) does not agree with her. As a matter of fact the specimens in which the change of shape is inhibited by anæsthetics show nothing of the sort even in regions adjoining those where new tissue is being formed, but if such pieces be returned to water the cells of the parenchyme become very distinctly elongated or oriented in the direction in which elongation of the body is occurring, even though no new tissue is being formed at the time. In short the change in shape or arrangement of the parenchyme cells probably has nothing to do with active migration, but merely indicates the direction of the strain which produces deformation.

These histological features then, support and confirm the other observations, and all appear to show that the change in shape is primarily a deformation in consequence of strain rather than a complex physiological process.

There can be no doubt, however, as I have repeatedly pointed out that reactions of various kinds result from the strain and deformation : muscles and other tissues undoubtedly "adapt" themselves to the new relations of parts. In fact there is no apparent reason why the change of shape should not continue indefinitely, or at least until the elasticity of the tissues became involved, if nothing but the mechanical deformation took place. Undoubtedly "functional adaptation" to the altered strains occurs and this determines how far the change shall proceed. Sooner or later the tissues adjust themselves fully to the new conditions, *i. e.*, a condition of equilibrium is attained and change in shape ceases. Under the usual conditions this gives what we commonly call the "normal" shape, but I have shown above that under other conditions the shape may be different. No one would deny, I suppose, that the shape of cells is determined in many cases (*e. g.*, the polyhedral shape of blastomeres in many eggs, the polygonal outline of many epithelial cells, etc.) to a greater or less extent by purely mechanical factors. If this is the case it seems scarcely possible to deny that purely mechanical factors may be concerned in determining the shape and arrangement of parts in animals without hard structures and with tissues of a high degree of physical plasticity. That they are the only factors in such cases, or that because they are factors in some cases, they must be in others, I certainly do not and never have maintained.

In *Planaria* the width of the head is apparently an important factor in determining the width of the body behind it. The head itself is not involved to any appreciable extent in the change of shape. It furnishes, so to speak, a fixed point, or rather region at one end, and between this and the posterior end the change of shape occurs. Consequently, in short pieces, where the new head is small, the change of shape is very much greater than in long pieces, where it is much broader. In short pieces possessing the old head and consequently capable of rapid and frequent locomotion, the change occurs much more rapidly than in other pieces and the body often becomes much more slender proportionally than that of the "normal" animal and tapers more toward the posterior end. In pieces from the posterior region of the animal the chief region of attachment forms the largest part of the piece. Since attachment occurs by the lateral margins as well as by the tip of this region, the change of shape occurs most rapidly in the regions just behind the new head, for these are the regions where the greatest change in the direction of the strains occurs.

Summing up the data presented, the first point of importance is that no necessary connection exists between the change of shape and the redifferentiation or regeneration of parts : the two processes can be separated from each other almost completely in time, and are often separated in space. Secondly, it is possible to control the change of shape, to inhibit it, to stop it at any point, or to allow it to proceed, by controlling the locomotor activity. And finally, the tissues in regions undergoing change of shape show very distinct indications of physical deformation in the direction of the strains. The conclusion which seems to me to accord most closely with the facts as they now stand is that the change of shape is primarily a mechanical deformation resulting, at least in large part, from the strains which arise in locomotion, these strains being altered in direction and amount in pieces as compared with the whole animal. The final shape, *i. e.*, the cessation of change is probably determined by the physiological reaction of the differentiating or redifferentiating tissues to the altered conditions, and the consequent establishment of an equilibrium.

II. Function, Form and Regulation.

Since the appearance of my earlier papers on regulation certain reviews and criticisms of the work have appeared. From some of these, particularly from Driesch's review (Driesch, '05) I can only conclude either that I have failed to state my position clearly, or that the reviewers have not become sufficiently acquainted with my work to understand what that position is. Driesch, for example, has imputed to me certain views which I have not only never held, but which I have expressly repudiated more than once. And recently, in a brief reference to my work, Morgan ('07, pp. 373–4) has cited certain conclusions as mine, which are very different from those which I have reached. I have considered Driesch's criticisms elsewhere (Child, '07) and shall refer to these and other criticisms only incidentally. But a brief statement of my position with regard to certain features of the problems of form and regulation and with especial reference to mechanical factors, movement and use of parts and function in general seems desirable in connection with the new facts presented above.

1. *The Relation between Function and Form.*[1]

In a fully developed organ certain processes occur which are concerned with the maintenance of the organism as a whole. These processes may affect either the relations of parts to each other or the relations of the organism as a whole to the external world, or both. They are commonly designated as functions, *i. e.*, the adult organism may be regarded as a complex machine, which works or functions in a characteristic manner.

But the functions characteristic of the adult organs do not appear in development until a certain stage is reached and the organ possesses a certain structure. Apparently then, development up to the stage where function in the above sense begins is a process of machine-building. Roux's distinction between a formative and a functional stage in development expresses this idea.

But how is the machine constructed and what is the agency which constructs it? The material of which the machine is to be made must be acted upon, arranged, transformed, localized, differentiated, etc. Evidently this point of view necessitates the assumption of a special formative agent or agents of some kind. How shall we conceive this formative agent? Pangenes, determinants, formative substances, entelechies are some of the answers which have been given to this question. All these answers are much alike in that they regard the construction of the organism as a process analogous in some sense to the construction of a machine by human agency. When the machine is constructed it begins to function.

---

[1] Cf. Child, '06*a*, p. 402, '07.

This point of view seems to me to be essentially naive and anthropomorphic : moreover it is responsible for certain "Scheinprobleme" which have arisen from time to time, and for certain barren lines of research, particularly in zoölogy, which has been dominated by hypotheses of this general character to a much greater extent than botany.

To my mind life is inconceivable without some sort of function in some sort of structure ; a living structure functions as long as it is alive ; its function is its life. If this view is correct, the organism is functioning in some manner at all stages of its existence, from the earliest to the latest. Function in this sense is physiological activity of all kinds, all transformation and transference of energy.

It is a well established fact that the special functional activity of organs is very generally a factor in their development and differentiation beyond a certain stage, and in the maintenance of their characteristic form and structure after development is completed. In the absence of this functional activity the organ does not develop beyond a certain point and does not persist indefinitely. Functional hypertrophy, atrophy from disuse, functional adaptation, etc., are terms used to designate these relations between function and form.

But the real difference between earlier and later stages of development consists, it seems to me, not in the absence of function at one stage and its presence at another, but rather in the difference in character of function in the different stages. If a particular kind of function determines the structure and differentiation at one stage, and we know that it does this, is there not a logical basis for the belief that the functions which exist in other stages are also formative factors in those stages ?

In other words, is there not good reason to believe that every stage of development is directly or indirectly the necessary consequence of the functional activity in the preceding stage ? According to this view each stage of development is a machine in the broad sense and each is the product of the activity of a preexisting machine. External factors play a part, particularly in plants and in the simpler animals, in determining the character of the machine and its activity, but this fact does not essentially alter the case.

Viewed from this standpoint, development, from its earliest stages on, is just as strictly a functional process as functional differentiation or functional hypertrophy in the stricter sense. This view does not necessitate the assumption of any special formative factors different in character from functional processes as the factors behind or underlying development. The formative factors of each stage are the functional activities of the immediately preceding stage (plus external factors). The process of development of the organism is not essentially different from the process of maintenance after development. Indeed strictly speaking, development ceases only when death occurs. The germ cell is an organism possessing a certain structure and function, and this forms the starting point. The functional activity in this structure determines the next stage of development, *i. e.*, a change in structure and therefore a change in function. This process continues and at the same time gradually approaches a condition of physiological equilibrium.

This view of development is of course no more an "explanation" than is the assumption of an entelechy or that of determinants. As a point of view, however, and as a basis for attacking the problem of morphogenesis it possesses a certain value in that it does away with various assumptions and places the problem of morphogenesis on a strictly physiological basis. To say that all organic form and structure are functional in their origin is merely to say that the problem of morphogenesis is a physiological problem and nothing more.

This is the position which I have held since the beginning of my work on regulation. I have used the word "function" with reference to any and all physiological processes and activities at any and all stages of development and have repeatedly called attention to this fact.[1]

---

[1] It is somewhat surprising, therefore, to find Driesch ('05, p. 790; '07, p. 180) pointing out that I have put the cart before the horse in regarding function as a determining factor in form regulation, since, as he says, an organ develops "for function" and does not function until it is developed, or at least until a certain stage of development has been reached. The difference between Driesch and myself on this

When we come to consider the process of development somewhat more in detail we find that two complex groups of internal factors must be considered, viz., constitution and correlation. This, of course, is true of any machine: its function depends upon the constitution and correlations of its parts, provided, of course, that the necessary external conditions for function are present. In different organisms and in different features of development the two factors may of course play a very different part.

2. *Functional Regulation and Form Regulation.*

It follows from what has been said that all form regulation is, according to my definition, either directly or indirectly functional regulation, *i. e.*, the physiological processess in the structure involved determine what the result of regulation shall be (Child, '06*c*).

In certain cases among the turbellaria I have been able to control, inhibit and modify the process of form regulation to a greater or less extent by controlling and modifying, in most cases indirectly, the movement and use of the parts most intimately involved in the regulatory processes (Child, '02, '03, '04*a–c*, '05*a–d*). This work showed very clearly that movement and use of parts were important factors in certain cases and certain features of regulation: they may even be primary factors in determining certain results. It is just as certain, however, that in many other cases they play no part at all.[1]

---

point is in reality merely one of definition; he limits the term "function" to the processes of which I spoke in the first paragraphs of this section, while I regard all processes in the organism as functions. His criticism of my position is of course entirely beside the point since he substitutes his definition for mine. Physiological processes of some kind certainly occur even in the earliest stages of development, and that these are functions of an existing structure cannot be doubted. I believe that they are also the formative factors in development.

Morgan ('07) calls attention to the fact that the functional idea is not new. This of course is true; it is the position which every physiologist must hold in one form or another. I have never considered that I was formulating a new hypothesis of development; I have merely attempted to apply certain physiological ideas to the phenomena of form regulation. Among botanists this view has been very generally held for a long time.

[1] Morgan ('07, p. 374) apparently believes that I regard movement as a universal factor in form regulation, for he calls attention to the fact that movement does not occur in many cases of regeneration. But I have repeatedly asserted that my conclusions upon the effect of movement concerned only certain species and certain features of regulation (Child, '02, p. 218, p. 229; '04*a*, p. 131; '04*b*, p. 467, etc.).

As I pointed out concerning *Leptoplana* (Child, '04*a*) movement is undoubtedly not a factor of importance in determining the formation of new tissue at a cut surface, but experimental evidence indicates clearly enough that it is a factor in determining the rapidity, amount and direction of growth and to a greater or less extent the character of its differentiation.

In the experiments on *Planaria* described above, the head and pharynx, for example, do not attain their "normal" shape and structure when movement is largely inhibited by anæsthetics. By the use of the higher concentrations it is possible to inhibit in almost any desired degree the process of form regulation so far as visible morphogenesis is concerned. Pieces which in water form heads very rapidly may be prevented entirely from forming heads by the proper use of the anæsthetic, or almost any intermediate condition between these two extremes may be attained by the formation of incomplete or partial heads. Pieces incapable of forming heads in the anæsthetic, regain their original power when returned to water. But it does not in the least follow from these facts that head formation is entirely the result of actual movement. My position is, and has been, that movement is merely one of the functional activities concerned in development, and which usually concerns later stages to a greater extent than earlier. But in regeneration in the turbellaria and in various, though by no means all, other forms a peculiar condition exists in that new tissue adjoins fully developed parts which may be in active movement. There can be no doubt that movement of these adjoining old parts influences conditions in the new tissue and so affects the result, either quantitatively or qualitatively or both. But movement and functional activity in the stricter sense, in which Driesch uses the word, are certainly far from being universal factors in either form regulation or ontogeny.

Whatever the importance of movement in a particular case, it is merely one of a great variety of functional factors. In many cases movement and motor use of parts are not concerned at all in regulation and in some other cases it is evident that while movement may affect the later stages this movement is possibly only in consequence of preceding regulation. In *Cestoplana* (Child, '05*b*), for example, where the posterior portion of an anterior piece becomes visibly "posterior" as regards the character of its movements before any marked structural changes occur, regulation must have occurred before such changes in movement could appear.[1]

3. *Mechanical Regulation and Morphallaxis.*[2]

In the first of the "Studies" the idea of mechanical regulation as the chief factor in the change of shape in pieces of *Stenostoma* was developed. According to my conception mechanical regulation is primarily a mechanical deformation of physically plastic tissues. Whether we consider regulation as a return or approach to the normal condition after a disturbance of this condition (Driesch, '01), or as a return or approach to a condition of physiological equilibrium after a preceding condition of equilibrium has been disturbed (my own definition), mechanical regulation in my sense is theoretically possible, provided certain characteristic mechanical conditions exist in the normal animal and are altered in characteristic manner in the piece. In several of the "Studies" the changes in shape in pieces of *Stenostoma*, *Leptoplana* and *Cestoplana* were shown to be apparently primarily mechanical regulations (Child, 02, '04*a*, '05*a*, '05*c*) and it was suggested that similar processes might occur elsewhere. The recognition of mechanical regulation is not in any sense an attempt to interpret regulation in general on a mechanical basis (cf. Child, '02, p. 229): it concerns merely changes in shape and outline. Mechanical regulations are possible only under the conditions mentioned above, and whether they occur or not can only be determined by investigation of each particular case. It is not necessary to suppose that all cases of "morphallaxis" (in the sense of change in shape and proportions) are mechanical regulations. If they are they certainly depend on different mechanical conditions in different cases. In *Hydra*, for example, the factors determining the change in shape are certainly not the same as in *Planaria*, and may not be mechanical at all. In the cases which I have considered mechanical strains arise in connection with locomotion and these strains are altered in a characteristic manner in isolated pieces and, as I have shown, the changes in shape are exactly what might be expected in physically plastic material under these conditions. Mechanical regulation is to be expected only in organisms or parts possessing a considerable degree of physical plasticity and where skeletal structures are not concerned. As regards the shape of animals in general gross mechanical factors are certainly unimportant as compared with others, or not concerned at all, and I have never even suggested that such factors were of general significance (cf. Driesch, '05, p. 790). On the other hand, it seems to me absurd to deny that characteristic strains or pressures existing in plastic material may play some part in determining shape.

---

[1] In my paper I referred to these changes as functional regulation and called attention to the altered character of the movement as indicating that they had occurred, but while the movement is undoubtedly a factor in what follows, it would be absurd to suppose that it is the primary factor in such a case.

[2] Some years ago, in describing the course of regulation in *Planaria maculata*, Morgan called attention to the peculiar changes in shape which the pieces undergo, these changes consisting mainly in a decrease in width and an increase in length. Concerning this process he says: "Thus the relative proportions of the planarian are attained by a remodelling of the old tissue. I would suggest that this process of transformation be called a process of morphallaxis" (Morgan, '98, p. 385). Later ('00) he applied the same term to the changes in shape and proportion in *Hydra* and other forms. So far as I am aware, Morgan has not stated at any time whether the term "morphallaxis" is to be applied to the whole process of form regulation in *Planaria* and other forms, including the regeneration and redifferentiation which occurs, or only to the changes in shape and proportions of the piece. From the quotation given above it would appear that he intended it to apply only to the change in shape and proportions, but in his latest statement ('07, p. 15) he apparently applies the term to the whole process of form regulation by redifferentiation. In my earlier "Studies" I used the term with reference only to the changes in proportion and shape: later, however, I substituted "change in proportions" for it as less ambiguous for my purposes (Child, '05*a*, p. 253). Driesch ('01) considered the term as synonymous with "Restitution durch Umdifferenzierung," a very different meaning from that which I had given it in my work. It is probable that Driesch's misunderstanding of my conclusions concerning form regulation is in part due to our different interpretations of this term.

In several of my earlier papers the word "form" was used for "shape" and "outline." I agree with Driesch that this use of the word may be misleading, but I was careful to distinguish between form in this sense and structure, and pointed out that the factors concerned with change in form probably had nothing to do in many cases with change in structure (Child, '02, p. 218).

That the changes in shape in pieces of *Stenostoma*, *Leptoplana*, *Cestoplana*, and *Planaria* are not primarily "functional adaptations" (cf. Driesch, '05, p. 766) is, I think, evident. A functional adaptation, as I understand it, is a change in structure which involves a change in functional capacity, either an increase or a decrease or a modification in kind according to the character of the factor inducing the change. The change in shape in the pieces of *Planaria*, etc., does not in itself involve any such change in functional capacity, moreover, functional adaptations to mechanical tension and pressure consist, usually if not always, in an altered resistance to the mechanical factor, not in a change of shape which accords with the laws of mechanics. The change in shape in these turbellaria is essentially similar to what would occur under the same conditions in any material of similar physical constitution and there is not the slightest evidence that it results from a change in functional capacity.

There is little doubt, however, that functional adaptations result from the change of shape: as was suggested above, it is probable that the cessation of the change and the final attainment of a more or less characteristic shape, *i. e.*, a condition of equilibrium, is in part the consequence of the functional reaction of the differentiating and redifferentiating tissues to the altered mechanical conditions. In other words, the change of shape brings about the functional adaptation instead of resulting from it.

But even though these changes in shape do not appear to be functional adaptations in Driesch's sense, they are functional regulations in my sense, and probably the simplest possible kind of functional regulation.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
March, 1909.

BIBLIOGRAPHY.

Child, C. M.

'02 Studies on Regulation. I., Fission and Regulation in Stenostoma. Arch. f. Entwickelungsmech., Bd. XV., H. 2 and 3, 1902.

'03 Studies, etc. II., Experimental Control of Form-Regulation in Zoolds and Pieces of Stenostoma. Arch. f. Entwickelungsmech., Bd. XV., H. 4, 1903.

'04a Studies, etc. IV., Some Experimental Modifications of Form-Regulation in Leptoplana. Journ. Exp. Zoöl., Vol. I., No. 1, 1904.

'04b Studies, etc. V., The Relation between the Central Nervous System and Regeneration in Leptoplana: Posterior Regeneration. Journ. Exp. Zoöl., Vol. I., No. 3, 1904.

'04c Studies, etc. VI., The Relation, etc. Anterior and Lateral Regeneration. Journ. Exp. Zoöl., Vol. I., No. 4, 1904.

'05a Studies, etc. VII., Further Experiments on Form-Regulation in Leptoplana. Journ. Exp. Zoöl., Vol. II., No. 2, 1905.

'05b Studies, etc. VIII., Functional Regulation and Regeneration in Cestoplana. Arch. f. Entwickelungsmech., Bd. XIX., H. 3, 1905.

'05c Studies, etc. IX., The Positions and Proportions of Parts During Regulation in Cestoplana in the Presence of the Cephalic Ganglia. Arch. f. Entwickelungsmech., Bd. XX., H. 1, 1905.

'05d Studies, etc. X., The Positions and Proportions of Parts During Regulation in Cestoplana in the Absence of the Cephalic Ganglia. Arch. f. Entwickelungsmech., Bd. XX., H. 2, 1905.

'06a Contributions Toward a Theory of Regulation. I., The Significance of the Different Methods of Regulation in Turbellaria. Arch. f. Entwickelungsmech., Bd. XX., H. 3, 1906.

'06b The Relation Between Regulation and Fission in Planaria. Biol. Bull., Vol. XI., No. 3, 1906.

'06c The Relation Between Functional Regulation and Form Regulation. Journ. Exp. Zoöl., Vol. III., No. 4, 1906.

'07 Some Corrections and Criticisms. Arch. f. Entwickelungsmech., Bd. XXIV., H. 1, 1907.

Driesch, H.

'01 Die Organischen Regulationen. Leipzig, 1901.

'05 Die Entwickelungsphysiologie von 1902–1905. Ergebnisse der Anat. und Entwickelungsges., Bd. XIV. (1904), 1905.

'08 The Science and Philosophy of the Organism, Vol. I., London, 1908.

Morgan, T. H.

'98 Experimental Studies of the Regeneration of Planaria maculata. Arch. f. Entwickelungsmech., Bd. VII., H. 2–3, 1898.

'00 Regeneration in Planarians. Arch. f. Entwickelungsmech., Bd. X., H. 1, 1900.

'07 Regeneration. Uebersetzt von M. Moszkowski. Leipzig, 1907.

Steinmann, P.

'08 Untersuchungen über das Verhalten des Verdauungssystems bei der Regeneration der Tricladen. Arch. f. Entwickelungsmech., Bd. XXV., H. 3, 1908.

Stevens, N. M.

'07 A Histological Study of Regeneration in Planaria simplicissima, Planaria maculata and Planaria morgani. Arch. f. Entwickelungsmech., Bd. XXIV., H. 2, 1907.

Woodworth, W. McM.

'97 Contributions to the Morphology of the Turbellaria. II., On Some Turbellaria from Illinois. Bull. Mus. Comp. Zoöl. Harvard Univ., Vol. XXXI., No. 1, 1897.

SOME ABNORMALITIES AND REGENERATION OF PLEIOPODS IN CAMBARUS AND OTHER DECAPODA.[1]

CHARLES ALBERT SHULL.

Introduction.

The two crayfishes whose abnormal appendages are described in this paper were found among a small number of specimens of *Cambarus virilis* Hagen, which were captured near the city of Chicago in the autumn of 1904. The discovery of these abnormal pleiopods led to an experimental study of the regeneration of the abdominal appendages in the crayfish, the results of which are given herein. The earlier experiments were unsuccessful owing to imperfect methods of handling the material, and to the use o mature animals in which the moults were infrequent. The later experiments, with improved methods of caring for the material, and with small immature specimens, have yielded satisfactory results.

These experiments were begun in the Hull Zoölogical Laboratory of the University of Chicago, continued in the Biological Laboratory of Transylvania University, and completed in the Marine Biological Laboratory of the Brooklyn Institute of Arts and Sciences at Cold Spring Harbor, Long Island. I wish to express my thanks to Dr. Davenport, director of the Marine Laboratory, who kindly placed the facilities necessary for the completion of the work at my disposal; also to Dr. A. E. Ortmann, of the Carnegie Museum, Pittsburgh, to whom I am indebted for identification of the species used in the experimental work; and to Dr. Faxon, of Harvard, and to Dr. Hans Przibram, of Vienna, for examining the abnormal specimens.

Historical Summary.

The regeneration of the abdominal appendages of decapod crustaceans has been a subject of investigation for many years, but not until recently has a critical study of it been attempted. A review of the earlier literature relating to regeneration in the Decapoda has been given by Miss Steele, '04; it is necessary, therefore, to mention only a few of the later papers upon whose results my own observations have some bearing.

---

[1] Contributions from the Biological Laboratory of Transylvania University, No. 2.

Morgan, '98, desiring to test Weismann's hypothesis, that regeneration is an adaptation, experimented with *Eupagurus longicarpus* to determine what relation, if any, existed between the power of regeneration and liability to injury. Because the hermit crab lives in shells where the abdominal appendages are protected from injury, whereas the thoracic appendages are not so protected, these two series of organs were chosen for this experiment. He came to the general conclusion that no such relation existed, and further supported this view by a second paper in 1900. The latter paper was based upon experiments in which the thoracic appendages were removed at unusual levels.

He found in the course of his experiments that the abdominal appendages of *Eupagurus* did not regenerate readily, although slight regenerative power existed. He suggested that this rarity of regeneration "may be connected in some way with the amount of food supply brought to the region from which they arise."

Miss Steele, '04, experimenting with *Cambarus virilis* and *C. gracilis* has obtained results similar to Morgan's. She says, speaking of the swimmerets: "I have found none to regenerate except the first pair in the male. . . . In the case of the other abdominal appendages except the sixth pair, regeneration, if it does take place, is very slow in beginning."

Emmel, '04, reported the observation of regeneration in the first appendages of the lobster. He experimented with the other appendages and says: "In experiments with the other four pairs of abdominal appendages or swimmerets, positive results were obtained in the second and third pairs, and it seems safe to say that all the swimmerets will regenerate."

Haseman, '07, mentions the regeneration of swimmerets in *C. propinquus*, giving figures showing the progress of differentiation, but he does not mention the fact that the regeneration of these appendages was considered doubtful.

A discussion of the results obtained by Morgan and Miss Steele will be found in connection with the discussion of my own observations in a later portion of this paper.

Abnormal Swimmerets.

Anomalous variations not of congenital origin are of little interest to the student of evolution; for it has never been definitely demonstrated that any acquired somatic variation is inherited. Moreover, no very important laws of variation are likely to be discovered by a study of teratological specimens. Nevertheless, it is advisable to place interesting cases of abnormal growth and regeneration on record, for we may finally acquire a knowledge of the physiology and mechanics of development sufficient at least to explain the origin of such anomalous variations.

The swimmeret shown in Fig. 1 is the right appendage of the second abdominal somite of a female *C. virilis.* The pleuron is much deformed, as is readily seen if compared with the normal pleuron of the opposite side of the same somite. The coxopodite of the abnormal appendage is much larger than normal. From this enlarged basal piece arise two appendages, one of which, near the posterior side of the coxopod, seems on casual observation to be a beautiful case of duplicity. The parts are perfectly doubled from the proximal portion of the basipodite outward. The two pleiopods thus united are normal in every particular beyond the point of union, the posterior member being somewhat larger than the anterior. From the anterior portion of the large coxopodite, completely separated in point of origin from the double pleiopod, arises another appendage which is uniramous, but of a size and structure typical of the endopodite of the second pleiopod.

It is an interesting circumstance that the first pleiopod lying immediately anterior to the one just described is also abnormal. This swimmeret is shown in Fig. 2. The lower portion of the appendage is much enlarged, and at the proximal end of the basipodite, there is a posterior projection set with stiff hairs, reminding one somewhat of the structure of the coxopodite of the pereiopods.

The third abnormal swimmeret is from a male *C. virilis* which was found to have three pairs of appendages instead of two, modified for sexual purposes. . The right swimmeret of the pair is shown in Fig. 3, the left one being exactly like it. The character of the modification in this third pair is just the same as that of the second, except that the projection of the endopodite is smaller than on the second. The first and second pairs of appendages are perfectly normal. Moenkhaus, '03, has reported an exactly similar case in the same species, and so far as I know these are the only two cases on record.

The Problem, Materials, and Methods.

Being convinced that the abnormal swimmerets of the female *C. virilis* described herein were the result of regeneration after a somewhat extensive injury, and having before me the work of Morgan on *Eupagurus longicarpus*, and of Miss Steele on *C. virilis* and *C. gracilis*, I determined to perform a series of experiments with the crayfish in order to ascertain whether or not the abdominal swimmerets regenerate, and if so, under what conditions. These experiments were begun in 1905; and after two years of rather unsuccessful work the choice of material and the methods of handling it were so improved that gratifying results have been obtained.

At first attempts were made to keep the crayfishes in aquaria of running water. A large aquarium was divided by partitions of galvanized iron netting into a number of compartments, each about 30 cm. square. Each compartment was provided with gravel and flat stones, and fresh water from Lake Michigan was kept running constantly at a depth of about 5 cm.

The aquarium was cleaned frequently, and all precautions were taken to keep the animals in sanitary conditions; but in spite of the great care exercised, the crayfishes would die after several weeks of confinement. Since my first material was adult, and moulted therefore infrequently, death took place before any moults occurred. Many variations of these conditions were tried in an attempt to secure more favorable results, but without success.

During the last two years a method has been employed, which has obviated all difficulties, and has given entirely satisfactory results. Young specimens of *Cambarus* (*Bartonius*) *bartoni* Fabr., probably at the beginning of their second and third years were taken on March 24, 1907, from a small stream which crosses the Bryan Station pike about three fourths of a mile northeast from the city limits of Lexington, Ky. The specimens used are not like the typical *C. bartoni* of the eastern United States, but have a narrower areola, less spiny carpus, and a shorter but broader rostrum than the eastern form. Dr. Ortmann states that they agree with specimens described by W. P. Hay in the 20th Ann. Report of the Ind. Geol. Survey, 1895, after comparing the living specimens with those in the Carnegie Museum from Mitchell, Lawrence Co., Ind.

Each specimen was put into a tumbler with water not quite sufficient in amount to cover it. A small piece of mica schist coming not quite to the surface of the water, was placed in each tumbler so that the crayfishes could crawl upon it and expose themselves to the air at will. The tumblers were kept nearly covered by glass plates to prevent accidental desiccation. Care was taken not to cover the dishes entirely, as the air was found to become overcharged with carbon dioxide in a short time if so covered. The water was changed completely three or four times per day, and even oftener on very hot days ; for crayfishes seem unable to endure warm water for any great length of time.

The water used in these experiments was supplied by the city water works of Lexington. This water is filtered through large Jewell filters before being pumped into the water mains, and is exceptionally pure. The complete change of such water every few hours rendered the multiplication of bacteria or growth of other fungous plants rather difficult, and tended to prevent the accumulation of any sediment.

When it appeared that any fungous growth was forming upon the appendages and about the thoracic region, each crayfish was treated for a few minutes with a bath of copper sulphate. The vessels and stones kept in them were occasionally treated with the same solution. The copper sulphate was used with a strength of one part to a million, when the animals could be left in it for some time. Solutions much stronger than this, one part in ten thousand, can be used for a few minutes at a time with entire success in destroying bacteria and fungi, and without injury to the crayfishes, provided they are washed thoroughly several times in plenty of pure water after the bath to cleanse their gills of the copper sulphate.

Young crayfishes are voracious creatures, and need to be fed frequently. I have fed them every day with entire success, but they soon tire of a uniform diet. Several kinds of food were used; and by rotation, so that they never received the same kind of food on successive days, their appetites were retained. Fresh raw beef was given them twice a week, and raw potatoes and pieces of *Myriophyllum*, which was kept growing in the laboratory, were used as vegetable foods. The *Myriophyllum* was covered with slime, which was found to contain unicellular algæ, rotifers, nematode worms, annelids, such as *Æolosoma hemprichii*, and other kinds of small animals. This slimy material was especially esteemed, and probably most nearly represents their normal diet. This food material was left in the glass with the animals for an hour or two, after which the remains were removed and fresh water placed in the vessels.

Using these methods, I have kept them alive for months in perfect health, with rapid growth during the moulting season. Occasionally a death would occur among them, but these fatalities were always obviously due to special causes, not to any general defects in the methods employed.

After ecdysis the cast off exoskeletons were used in the preparation of the drawings.

Experiments.

The experiments here described are a few typical ones selected from a series, all of which gave similar results. The numbers correspond to those used while recording notes on individual experiments.

No. 4. *C.* (*Bartonius*) *bartoni*, ♀. Fifth right abdominal appendage removed March 24, 1907. The appendage was cut off, leaving a short stump attached to the body (Fig. 4).

The first moult occurred in the afternoon of March 27, three days after the operation. No regeneration could be noticed, but the wound was perfectly healed (Fig. 5). The second moult occurred during the night following May 6, 1907. Between March 27 and May 6 no change could be detected even by use of the microscope. But that changes were taking place beneath the chitinous cuticle is evident from the expansion of the regenerating limb, which occurred immediately after this second ecdysis (see Fig. 6).

A third moult took place on June 19, 1907, at which time the appendage again expanded, reaching the size and condition shown in Fig. 7. The regenerated appendage measured at this time 3.3 mm. in length; the one on the opposite side of the same somite 4.8 mm. (Fig. 8). The regeneration was equal to nearly 69 per cent. of the normal size from March 24 to June 19, a rate which I consider by no means slow.

Owing to an unfortunate accident this crayfish was killed on the morning of July 5, 1907. Had it lived until after its fourth moult it would probably have shown a much more nearly complete regeneration of the appendage.

No. 7. *C.* (*Bartonius*) *bartoni*, ♀. Fourth left pleiopod removed March 24. The first moult after the operation took place the night of April 22, 1907. The amount of regeneration while not extensive was plainly visible. The second moult occurred May 25, 1907. The regenerating appendage expanded to nearly half the natural size, immediately after the exoskeleton was cast (Fig. 9).

No. 3. The same as No. 7 except that the fourth pleiopod was removed from the right side instead of the left. The first moult, on March 30, showed a small whitish papilla a little over one mm. in length, projecting from the base of the amputated limb. This projection increased slightly with age, but this individual was killed in July by the water in the vessel receiving direct sun-light through a partially open window blind, thus becoming much overheated. Fig. 10 reveals internal development. A later moult would probably have given results similar to No. 7. This specimen was probably a year, or perhaps even two years, older than No. 7, and consequently only one moult was recorded between March 24 and July 5, while some of the smaller, younger specimens moulted three times in a shorter period (cf. No. 4).

No. 2. *C.* (*Bartonius*) *bartoni*, ♂. The third right appendage was cut off March 24. On May 9, no moult having occurred, the stump of the appendage was cut longitudinally by means of sterilized scissors. The moult occurred on June 17, but no abnormal growth was noted. The appendage was perfect, and showed regeneration of more than 50 per cent. in size (Fig. 11).

No. 5. *C.* (*Bartonius*) *bartoni*, ♀. The second left appendage was removed March 24, 1907. The first moult occurred on May 8, 1907, and the regeneration amounted to about 30 per cent. The second moult took place on June 26, 1907, when the appendage showed about 65 per cent. of complete regeneration. Fig. 12 shows the point of amputation, and Fig. 13 the appendage after the second moult.

No. 6. *C.* (*Bartonius*) *bartoni*, ♂. Sixth right appendage was amputated just beyond the basal joint March 27, 1907, as shown in Fig. 14. It moulted the following day. A very narrow edge of white tissue was visible along the cut edges, but it is not probable that any regeneration had taken place. During the time preceding the next moult which occurred on May 27, the basal portions of the rami increased somewhat in size (Fig. 15), and after moulting the appendage was about one half natural size, and perfectly formed (Fig. 16).

A series of experiments on the pleiopods of *Palæmonetes vulgaris* was carried on at Cold Spring Harbor, and regeneration of all the abdominal swimmerets takes place rapidly in young specimens.

At present a series of experiments to test the regeneration of the antennæ from various levels and to compare the rate of regeneration of pleiopods and pereiopods in *C.* (*Bartonius*) *bartoni* is being carried on; but the data are insufficient as yet to permit a general statement regarding either phase of the series.

Discussion.

A. *The Abnormalities:* — As far as I have been able to ascertain, only one abnormal abdominal appendage has been recorded for any of the decapod crustaceans. In as much as all abnormal pleiopods described herein or elsewhere have been discovered accidentally, the rarity of such records is due in part, perhaps, to lack of careful observation; but the examinations which have been made show that they occur infrequently.

The abnormalities presented here are of interest for several reasons. The abnormal pleiopod is evidently the result of a regeneration after extensive injury in one instance, and just as evidently the result of undisturbed growth processes in the other instance. That is, the condition is congenital and may be considered as having arisen by mutation.

It would appear that the swimmerets shown in Figs. 1 and 2 probably resulted from an injury which removed part of the pleuron, and tore the first and second pleiopods near the base of each, as represented diagrammatically in Fig. 17. The coxopodite in Fig. 1 is abnormally large, which condition may possibly be explained by the fact that a large area was exposed when the mutilation occurred.

Very few genuine cases of duplicity have been described. Bateson mentions only four cases, all chelæ; Herrick has figured a double chela of a female lobster, and Zeleny has described a double chela of *Gelasimus pugilator* which regenerated instead of a normal single one during the course of his experiments.

Bateson states emphatically that "in arthropods and vertebrates such a phenomenon as the representation of one of the appendages by two identical appendages standing in succession is unknown. No right arm is ever succeeded on the same side of the body by another arm properly formed as right, and no crustacean has two right legs in succession where one should be."

While this supernumerary appendage may be regarded as a complementary image of the normal one, and therefore a left appendage instead of a right, there is nothing in the structure of either member to indicate that such a relation exists. The members are not imperfect, and are placed in succession, differing in these two respects from the cases admitted by Bateson.

At first appearance the coxopod of the abnormal first appendage is very much like that of the last pereiopod. And the anterior uniramous part of the second pleiopod is like the first pleiopod in being uniramous, but is a typical endopodite. There is a mere suggestion here of a shifting backward of the series of organs, a condition to which Bateson applied the term backward homœosis.

However if the injury actually took place as indicated in the diagram referred to above, the most plausible explanation which can be offered is in harmony with Bateson's statement. The regeneration should lead theoretically to triplication, that is, to the production of three appendages, two supernumerary ones on each cut appendage; and these on the right side of the body should stand as a left between two rights. The abnormal pleiopod shows defective regeneration only in the suppression of the exopodite of the anterior supernumerary appendage. The rounded projection of the first accessory pleiopod may be explained similarly as the fused basal portions of the two supernumeraries, whose tapering jointed ends have been completely suppressed.

The abnormalities have probably arisen after an injury caused by some force acting in the direction of the arrow in the diagram, Fig. 17, which produced two breaking surfaces, from which the new pleiopods arose, following the laws of symmetry for supernumerary appendages as stated by Bateson.

The abnormal appendage shown in Fig. 3, is a case of backward homœosis. It would be of interest to know the hereditary behavior of such unisexual characters if bred in confinement.

B. *Experiments.* — Few investigations of the regenerative power of the abdominal appendages of the Decapoda have been made, and the results obtained have not been entirely satisfactory. Morgan's experiments on *Eupagurus longicarpus* in 1898 showed that a slight power of regeneration existed in the appendages of two or three of the individuals he used; but his experiments extended over too brief a period of time to secure any marked regeneration. The first experiment was continued only twenty days, and the second for twenty-eight days. And the conditions under which the material was kept were possibly not the most sanitary, as the fact that over 40 per cent. of the individuals died during the twenty-eight days would seem to indicate. One would hardly expect regeneration to be rapid under conditions in which life itself could scarcely be maintained. My experience has shown that although regeneration may occur after an operation, and become visible without an intervening moult, it usually does not do so in the pleiopods. None of the individuals Morgan used was kept until a moult had occurred.

Miss Steele's experiments on *C. virilis* and *gracilis* were carried on for a sufficient length of time, but she found it difficult to provide the sanitary conditions necessary to such prolonged experimentation. The specimens used in her work were probably too old to give the best results. They measured not more than three inches in length, a size which would indicate that they were several years old at least. The younger the specimens used, the more frequently the moults take place. Those used in my experiments measured from one and six tenths to two and five tenths cm. in length, and were probably at the beginning of the second or third year. Two individuals measuring five cm. in length were also used. These did not yield as satisfactory results although some regeneration occurred. The difference was probably more in the frequency of moulting than in anything else. While one moult took place in these older ones, I could secure three in the younger. And to have kept the older individuals until the same number of moults occurred as in the younger ones, would have required two years instead of four or five months. The use of more nearly adult material, and the fact that slight attention was paid to the swimmerets may account for the slight regenerative power which Miss Steele was able to report.

My experiments show that the swimmerets of young specimens of *C.* (*Bartonius*) *bartoni* regenerate rapidly, and that the regeneration of any of the appendages may be practically completed in a single season of growth. The regeneration is not particularly slow in beginning, having become visible in one instance only six days after the operation was performed. It is usual, however, to find that the regeneration begins and takes place under the old exoskeleton, without showing any visible indications that the new parts are forming. For instance, No. 9 moulted on March 28, four days after the operation, and no regeneration could be noticed. The next moult took place April 30, but during that time no indication whatever that regeneration was occurring could be seen. Nevertheless, when the moult occurred it was evident that regeneration had taken place. If the experiment had been continued for only thirty days, the conclusion naturally drawn would have been that regeneration either did not occur, or was " very slow in beginning." As a matter of fact there is no way to tell how soon after March 28 the regeneration did begin, if indeed it had not already begun at that time.

To explain the slight regenerative power which he found in the abdominal appendages of *Eupagurus*, Morgan suggested that the food supply of these organs might be considerably less than that of the thoracic legs. It seems to me quite unnecessary to make this assumption, especially since I have shown that there is a rapid and complete regeneration of the swimmerets in young specimens of *C.* (*Bartonius*) *bartoni.* In this connection Emmel, '04, observed that swimmerets in the lobster will regenerate more rapidly than the pereiopods if the latter are cut "only a relatively short distance below the breaking plane." And he questions whether the supply of food material can explain the comparative difference in the regeneration of pereiopods and swimmerets. Moreover, if the limited food supply is responsible for lack of regenerative power how must we regard the regeneration of two supernumerary appendages in the abnormal swimmeret figured? It seems to me that some other explanation must be offered for the difference in regenerative power.

It has been a common experience with those who experiment with regenerative tissues, that the regeneration is always more rapid and complete in young individuals than in old ones. This fact is due probably to the greater plasticity, the more active and mobile condition of young tissues. They are more nearly embryonic in character, differentiation is not so complete, nor so fixed as in the older tissues. Emmel's ('08) recent work on the reversal of asymmetry in the lobster lends emphasis to this statement. He says: "In the first four stages of the lobster's development, a crusher may be produced on either the right or the left side of the body by the autotomous amputation of the chela on the opposite side — the regenerated chela becoming a nipper. During the fifth stage, although the chelæ are still symmetrical, the possibility for such experimental control disappears."

Since differentiation is known to proceed at different rates in different parts of the body, we may suppose that one part retains its primitive condition longer than another. If any portion of the adult regenerates less rapidly than another portion, may it not be an index of the comparative plasticity of the two parts? The more rapid regeneration of the thoracic appendages than of the swimmerets in the adult crayfish, may be due to a longer retention of the plastic embryonic condition of the cells in the region of the breaking joint by the former, certainly not to any proportionate difference in the food supply of the two series of organs.

The important point in Morgan's results which he properly emphasized, was that regeneration did take place, however slight it might be. While we cannot say that the abdominal appendages of crayfishes are never injured, yet injuries to them are rare in nature. Examination of hundreds of individuals has shown that there is a remarkable uniformity of size and structure in these organs, a condition which could not exist if mutilations were frequent. Yet these appendages possess as high a power of regeneration in youthful stages as any of the appendages which are so frequently torn away. My experiments strengthen very much the evidence in favor of Morgan's statement that there is no relation between power of regeneration and liability to injury.

Conclusions.

The following conclusions may be drawn from the study of abnormalities, and the results of the experiments described in this paper.

1. The abdominal swimmerets of *C.* (*Bartonius*) *bartoni* Fabr. all possess a high power of regeneration in immature specimens. Since regeneration of swimmerets has been noted in *Cambarus propinquus*, *Palæmonetes vulgarus*, *Homarus americanus* and *Eupagurus longicarpus*, it is probable that the decapods generally possess this power, especially in young individuals.

2. This regeneration usually cannot be seen till after one, and sometimes two, moults have occurred, due to masking of the regeneration by the exoskeleton.

3. Slow regenerative processes in the swimmerets of the older individuals are due probably to a lower degree of plasticity in the protoplasm rather than to insufficiency of the food supply.

4. Injuries may occur, but they are rare in swimmerets, and the power of regeneration and liability of the parts to injury are apparently independent.

LITERATURE CITED.

Bateson, W.

'94 Materials for the Study of Variation. London, 1894.

Emmel, V. E.

'04 The Regeneration of Lost Parts in the Lobster. Thirty-fifth Annual Report of the Commissioners of Inland Fisheries of Rhode Island, pp. 81–117, 1904.

'08 The Experimental Control of Asymmetry at Different Stages in the Development of the Lobster. Jour. of Exp. Zool., pp. 471–484, 1908.

Haseman, J. D.

'07 The Direction of Differentiation in Regenerating Crustacean Appendages. Archiv für Entwicklungsmechanik der Organismen, pp. 617–637, 1907.

Herrick, F. H.

'95 The American Lobster. Bull. of U. S. Fish Comm., 1895.

Hay, W. P.

'95 The Crawfishes of the State of Indiana. Twentieth Ann. Report of the Indiana Dept. of Geol. and Natural Resources, pp. 475–507, 1895.

Moenkhaus, W. J.

'03 An Extra Pair of Appendages Modified for Copulatory Purposes in Cambarus virilis. Indiana Univ. Bull., Vol. I., No. 4, pp. 17–18, 1903.

Morgan, T. H.

'98 Regeneration and Liability to Injury. Zoöl. Bull., I., 1898.

'00 Further Experiments on the Regeneration of the Appendages of the Hermit Crab. Anat. Anz., XVII., 1900.

Steele, M. I.

'04 Regeneration of Crayfish Appendages. Univ. of Mo. Studies, Vol. II., No. 4, 1904.

Zeleny, C.

'05 The Regeneration of a Double Chela in the Fiddler Crab (Gelasimus pugilator) in Place of a Normal Single One. Biol. Bull., Vol. IX., pp. 152–155, 1905.

Explanation of Plate I.

All figures except Fig. 17 are × 5.

Fig. 1. Second right pleiopod of *C. virilis* Hagen, ♀, showing two supernumerary appendages.

Fig. 2. First right pleiopod of the same specimen.

Fig. 3. Third right pleiopod of *C. virilis*, ♂, modified for sexual purposes.

Figs. 4–7. Successive steps in the regeneration of the fifth right pleiopod in *C.* (*Bartonius*) *bartoni*, ♀.

Fig. 8. Left pleiopod from same somite as Fig. 7, without regeneration, drawn to same scale.

Fig. 9. Fourth left pleiopod of young *C.* (*Bartonius*) *bartoni*, ♀, after two moults.

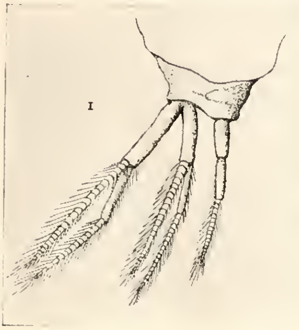
Fig. 10. Fourth left pleiopod of older *C.* (*Bartonius*) *bartoni*, ♀, after three and one half months.

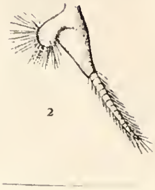
Fig. 11. Third right pleiopod of *C.* (*Bartonius*) *bartoni*, ♂, after one moult.

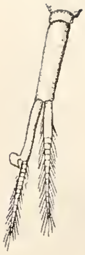
Figs. 12–13. Second left pleiopod of *C.* (*Bartonius*) *bartoni*, ♀, condition immediately after amputation, and after the second moult.

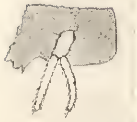
Figs. 14–16. Right uropod of *C.* (*Bartonius*) *bartoni*, ♂, condition at amputation, before, and after, second ecdysis.

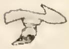
Fig. 17. Diagram illustrating the way in which the abnormal appendages shown in Figs. 1 and 2 may have been produced.

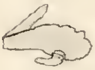

1

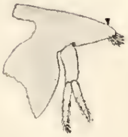

2

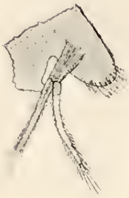

3

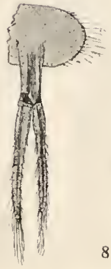

9

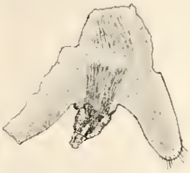

4

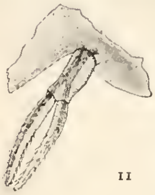

5

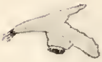

6

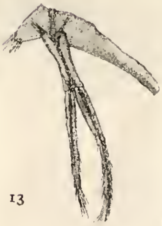

7

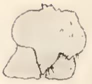

8

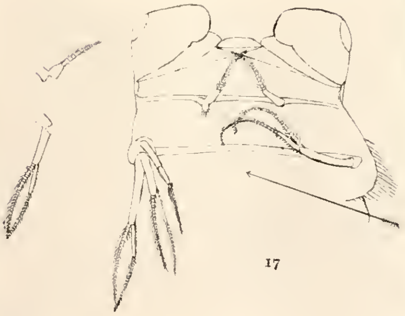

10

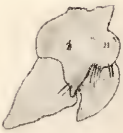

11

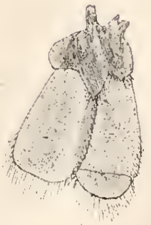

12


13


14


17


15


16

SEX RECOGNITION IN CYCLOPS.

S. J. HOLMES.

The sexual behavior of copepods presents several points of similarity with that of the amphipods which was described by the writer in a previous paper.[1] In both groups the males clasp and swim about with the females for a long time previous to copulation; and in both groups the behavior of the female is much the same while being clasped by the male. Having an opportunity to study a thriving culture of *Cyclops fimbriatus* in which pairing was actively going on the endeavor was made to ascertain if the method of the sex recognition employed in the amphipods occurs also in this species of a quite distantly related group.

Male *Cyclops*, as is well known, have the first antennæ enlarged and modified to form a clasping organ. In *Cyclops fimbriatus* the male usually clasps the female just in front of an enlargement at the base of the abdomen. Females carrying eggs are sometimes seized, and also females not more than half grown. Males show great eagerness in grasping the females, and they can be compelled to release their hold only with difficulty. They may be poked about roughly with a needle and the posterior part of the body may be cut off without causing them to leave the female. I have often picked up pairs in a fine pipette and forcibly squirted them out several times without succeeding in separating the two sexes.

As the pairs of *Cyclops* swim through the water the males are usually the more active. Frequently the female remains entirely quiet with the appendages drawn close to the body, and the body flexed vertrally, allowing herself to be passively carried about by her mate. At other times the female may swim as actively as the male. In general the behavior of the females and their attitude while being carried closely resemble what is found among the Amphipoda. So also does their behavior when the males come in contact with them and attempt to seize them. The female during the efforts of the male to clasp her around the base of the abdomen usually lies quiet with the appendages drawn close to the body. She may be seized by the legs, tip of the abdomen, or any other part of the body, but the male works around until he gets into his normal position which he sometimes attains only after much labor. Females vary greatly however in respect to their willingness to be clasped by the male, certain individuals resisting seizure for a long time.

---

[1] Biological Bulletin, Vol. 5, 1903.

So far as could be detected the males do not seek or follow the females at a distance as Parker concluded they did in *Labidocera.* The association of the sexes seems to be due merely to chance collisions. Males often attempt to seize other copepods with which they collide regardless of their sex. The males resist such attempts at seizure and dart quickly away, while the females often stop and submit readily to the clasping propensities of their companions. Several males were injured so that they could not resist seizure, and in many cases they were seized by other males who worked industriously until they got their burden clasped around the base of the abdomen in the usual way. These associations did not last long however; the active males apparently appreciating that something was wrong soon swam away. Recently killed females were often seized and in some cases carried about for a while, but they were finally dropped. Males seem rather more prone to seize dead females than members of their own sex. In one case I saw three males tugging away at a dead female, and they were soon joined by a fourth male who participated in the same effort.

It is possible that the odor of the female determines to a certain extent the sexual behavior of the males, but my experiments yielded no evidence of this. Several females were put into a tube one end of which was covered with fine gauze and the tube was then placed obliquely in water in which were numerous males. The males showed no tendency to congregate around the end of the tube where the females were confined. In another experiment several females were placed in a glass tube in which a small plug of loose cotton was inserted a short distance from one end. This end was laid obliquely in the water. The males showed no tendency to enter the open mouth of the tube as they might be expected to do if they were attracted by the odor of the females. The experiment of removing the organ of smell which was performed in the case of the amphipods would be a fruitless one in *Cyclops*, as the seat of smell is located to a considerable degree at least in all probability in the same organs that are used for clasping.

It is evident that mating in *Cyclops* is brought about much as it is in the Amphipoda. The males have a strong tendency to clasp other copepods ; the females tend to remain quiet in a condition somewhat resembling the death feint while being seized by the males. It is not improbable that olfactory stimuli may cause the males to remain with the females longer than they otherwise would, and they may render the males rather more prone to seize females than other males, but so far as could be determined by watching the behavior of the animals the specific reaction of the two sexes to certain kinds of contact stimuli is the main factor in bringing about their association.

OUR KNOWLEDGE OF MELANIN COLOR FORMATION AND ITS BEARING ON THE MENDELIAN DESCRIPTION OF HEREDITY.[1]

OSCAR RIDDLE.

Hardly a year has passed since the rediscovery of Mendel's Law without several additions to its descriptive terminology. This may signify either one of two things: a very healthy and vigorous growth, or the onset of senescence. Some of these newly introduced features are plainly justifiable; but there is reason to believe that the rather long series of extensions which has been made in recent times carries as its result not so much description of fact, as of deduction and far-reaching theory.

The facts and phenomena discovered by Mendel, and the array of facts of high importance which later workers in this field have brought before biologists, have already proved their value. The proved value of these facts is, however, no proof of the correctness of Mendelian interpretations of the processes of inheritance. I shall here present some *facts* which seem to indicate that these Mendelian *interpretations* are not sound; and further, that these unsound interpretations now stand as a formidable block in the path of progress to a better knowledge of the mechanism of inheritance and development.

Mendelian workers think that they have discovered, and certainly they have named and labelled, many "factors"[2] as necessary for the production of some single characters. These workers tie *all* of these factors together, and — for them — together they go into the germ cells, and whatever appears — or fails to appear — in the zygote is interpreted in terms of the

---

[1] Read January 19 before the Biological Club of the University of Chicago.

[2] The word *factor* as used here in a purely Mendelian sense represents quite a different thing from the physiological sense of the same word. The whole series of ordinary environmental factors — temperature, light, reaction of medium, concentration, moisture, etc., all these would not constitute even *one* Mendelian "factor." The Mendelian "factor" is often a rather unidentifiable thing, but it is conceived of as something capable of residence in, and of segregation by, the germ cells.

presence or absence (*dominance, inhibition, contamination*, etc., made use of by some workers) of *particular factors in the gamete.* But facts at hand, despite an opposite contention, will go very far towards showing that the method of analysis of the Mendelian worker has not permitted him to decide the question as to the *real number* and *separateness* of the factors ; nor yet to determine as to whether certain of the factors were at all *represented in the germ cells*, or whether they may not have arisen during the ontogeny as a direct result of tissue differentiations, through regulatory processes, or otherwise, and quite independently of the existence of a definite determiner in the gamete or germ cells.

As Mendelism has developed, it has lent support to the doctrines of preformation, unit characters, and discontinuous variation. The facts and interpretations here brought forward disclose, on the other hand, no small amount of epigenesis, and strongly support the proposition that present and new knowledge will lessen, not widen, the apparent gap between discontinuous and continuous variability. There is, too, at present a marked tendency in some quarters to further elaborate and extend the "factor" hypothesis, which furnishes an additional and specific reason for my calling attention to some facts from my province of study which indicate that already we have represented too many factors in the germ cells ; that quite certainly some factors which have by Mendelian interpretation been made to circulate through the germ cells are never represented (in the Mendelian sense) in these cells at all ; and finally that many factors considered most separate and discreet by Mendelians, can now be proved to be but points in lines of perfect continuity.

It will no doubt be urged by some Mendelians that the observations recorded here are quite wide of the mark because the writer has no experience in animal breeding. It is very true that I have not personally carried through any breeding experiments whatever. For information in this field I have depended upon what I have been able to see of the breeding and hybridization experiments conducted by others, and upon the literature of the subject. My own work for several years has been largely in the field of developmental and color physiology ; its aim being to get at the basis of the color characters of organisms. It must rest with biologists generally, however, to decide whether the facts here presented have, or have not, to do with the Mendelian interpretation and description of the processes involved in heredity and development.

The basis, then, of my objections to much of the Mendelian interpretation rests upon chemical and physiological facts regarding the origin and development of melanin pigments. It is necessary to anticipate the query as to how, or by what right, has melanin color formation anything to do with the essential points of Mendelism? I realize fully that the line of contact between these two provinces of activity is apparently not a line of contact at all, and so new and untrammeled is the territory that one would almost hesitate to enter, had not a pair of such good Mendelians as Cuénot and Bateson already knocked importuningly at the gateway which leads into it.

It should be recognized at the outset that, in thus presenting a body of facts from one field, as having important bearing on facts and theoretical deductions in another field, there is every risk that a short presentation will be incomplete, inaccurate, and at the same time may fail to properly or sufficiently orient the reader with respect to the writer's point of view. It is here impossible entirely to avoid incompleteness, inaccuracy, and but partial explanation of an opposed interpretation of the facts of color development and inheritance; it is hoped, however, that a presentation, and rather general though cursory discussion, of a limited number of facts — facts with which most biologists are not familiar, and which have never before been treated in this connection — will make it possible to recognize that some points of present biological theory are involved.

And, though many of my statements concerning such points of theory may seem dogmatic, I should like to make it clear that I am not deceived or blind to the fact that my present function is merely to introduce subject matter for a chapter, not to conclude a volume; to propose, not to decide. These discussions of theory would have been omitted entirely from this paper if it had been thought that the facts here brought for the first time into the field of heredity and developmental physiology would receive the attention which they deserve without such a setting. I am, of course, rather confident of the correctness of the point of view set forth. I am absolutely convinced that the facts here presented will prove valuable assets to the student of development and inheritance.

There are three reasons why melanin color formation, better than any other process or group of processes, may furnish the starting point for certain inquiries and criticisms regarding the way Mendelian inheritance is construed and described:

1. Color characters have been more extensively studied and described from the Mendelian standpoint than have any others. A very considerable share of the color investigated — all mammalian color, for example — is due to melanin pigment.

2. It was to recognize a fact in melanin color development that Cuénot ('03) introduced the idea of *presence* and *absence* of a character, or character determiner, etc.; an idea which is now made by many workers to support practically the whole structure of Mendelian description and interpretation. Again, the now rather elaborate terminology introduced by Castle is based almost wholly upon the behavior of melanin colors. A few paragraphs of the paper by Cuénot furnish practically all there is of a tangible basis for representing *chromogens*, *enzymes*, and *activators* in gametic formulæ.

3. There is already at hand a certain amount of definite chemical knowledge, and some reasonably safe physiological information, which can be brought to bear on some points of the color philosophy of Mendelism. There is, moreover, something which though apparently less substantial, is none the less important — namely, the assurance of further, definite light from these same sources. There can be no doubt that we can use biochemical and physiological methods and data to give us what is now more needed than all else, perhaps, in the study of evolution and development — namely, *the intimate developmental history, and nature of some one character;* I mean the *proximate* history, the mechanics of what some would call the "late stages" of the development, or the "differentiation" of a character.

It may help to keep the reader oriented throughout this discussion to state that I shall first describe some of the facts of color development as they are known at present from chemical, pathological, and physiological experience; and afterward sketch very briefly the nature of the Mendelian terminology; this to be followed by some discussion of my point of view. Such facts of color will be considered as have bearing on the following points:

Do the known facts of the *genesis*, *nature* and *history* of color characters harmonize with, supplement, modify, or radically differ from, the demands of present Mendelian interpretation? Do they enable us to decide as to whether color characters are qualitative or quantitative in nature? Are color differences cases of continuous or discontinuous variability? Can these facts throw light upon the existence or nature of unit characters? What of the purity of gametes? Do these facts indicate a different or sounder basis for the interpretation of Mendelian, or other inheritance? What justification or light, if any, is thrown upon the present practices of (*a*) adding "factors" in order to account for the inheritance phenomena exhibited by a character; (*b*) of tying all these "factors" together and postulating that all pass (by means of their representatives) through the germ cells?

Some Facts of Melanin Color Formation.

In a consideration of the facts of the origin of melanin coloration, one might deal at some length with the *distribution* and *histogenesis* of melanin. Though several interesting and illuminating facts lie in each of these directions, I shall dismiss these two phases of the origin of melanin colors with the single statement that the melanins are usually dark, amorphous or granular pigments, chiefly of intracellular, animal origin; extending within this kingdom from the trypanosomes (Protozoa) to man. There is no vertebrate species (unless we may think of pure albinos as such), but has this coloring matter in one or several parts of its body. It is, however, the chemical and physiological phases of the origin of color that it is most desirable to discuss, and it is from this angle of approach that we find most of the facts which bear on the Mendelian description of heredity.

Our knowledge of what has been called the "mechanics of melanogenesis" may be thought of as having begun with studies in the production of artificial melanins, and the accompanying search for the (melanin) chromogen in the albumen molecule. This work was shared by many workers: Stadelmann ('90), Gmelin ('94), Nencki ('95), Schmiedeberg ('97), Chittenden and Albro ('99), Hofmeister (see v. Fürth, '04), v. Fürth ('99, '01, '04), Hopkins and Cole ('01, '03), Schneider ('01), Samuely ('02) and others. Through these workers it was made known, first, that melanins artificially produced are essentially the equivalents of natural melanins; and second, that tyrosin and related aromatic compounds are the chromogens concerned.

The second step in the progress of this knowledge was concerned with the nature of the process by which the melanin is formed from the chromogen. Hlasiwetz and Habermann ('73) had first recognized oxidative processes as necessary for the formation of the artificial melanins. Landolt ('99) extended this fact to the natural pigment of the choroid.

Bertrand then discovered ('96) an oxidizing enzyme — tyrosinase — which was able to transform tyrosin into melanin-like bodies. Bertrand found the enzyme in certain plants. It has since been found to be of wide distribution, having been found by Biedermann ('98) in the contents of the alimentary canal of meal worms; by Lepinois ('99) and Gessard ('01) in the adrenal glands; by Gonnermann ('00) in beet roots; by v. Fürth and Schneider ('01) in the hæmolymph of insects; by Przibram (see preceding, '01) in the ink-sacs of cephalopods (*Sepia*); by Ducceschi ('01) in the blood of *Bombyx*; by Gessard ('02*a*, '03*a*, '03*b*) in the ink-sacs of *Sepia*, in the integuments of insects, and in melanotic tumors of horses; by Dewitz ('02) in the blood of certain insects; by Durham ('04) in the skins of mammals and birds; by Weindl ('07) in the skin, eyes, ink-sacs and eggs of *Loligo*; and by Bertrand and Mutermilch ('07) in wheat bran. v. Fürth and Schneider ('01) concluded that "tyrosinase-like ferments are widely distributed in the animal organism, and probably always appear wherever and whenever a physiological or pathological formation of melanin occurs."

Meanwhile, another advance in our knowledge of melanogenesis was made when Dewitz ('02) demonstrated the rôle of an oxidizing enzyme (tyrosinase) in the normal development of the dark pigment of the integuments of living, growing animals (fly-larvæ — *Lucilia Cæsar*). At the same time he was able to prove that, in the forms with which he worked, free oxygen is also an indispensable factor in the development of the color. This work, important and suggestive as it was then, is now made still more valuable by new knowledge of the chromogen — that is, the other factor involved in the pigment formation. Without knowing just what this chromogen might be, Dewitz was able to conclude (p. 45), "We cannot doubt that we have here in the blood of the larvæ an enzyme under the influence of which a chromogen is oxidized and forms a brown or black pigment."

A year later Gessard ('03*b*) was able to show that in the melanotic tumors of white horses not only tyrosinase but free tyrosin is present. He concludes (p. 1088): "Tyrosin is then the chromogen, the oxidation of which by tyrosinase determines the formation of the black pigment which is common to many physiological and pathological products of the animal economy; and it can be said that the color of the negro is due to the same reaction that produces the ink of the squid, or the black color of some mushrooms." Gessard states, too, that when tyrosin is oxidized with tyrosinase it gives a series of colors — "rose, rouge-grenat et brune." In a later work ('03*d*) he made a closer study of the color reactions of tyrosin in which he showed that the presence of acids, alkalis and salts have marked effects on the colors produced.

The recent work (May, 1908) of Bertrand, is, however, of the highest interest. He has been able to determine (1) the *type of substance — of which there are many representatives — which can by the use of tyrosinase be oxidized to melanin compounds*; (2) *he has shown that each one of these compounds passes through a series of colors before arriving at the final stage of oxidation*; (3) *that this series varies somewhat as to the exact tint of the initial and final colors*, but that (4) the *early stages of oxidation uniformly give lighter colors than the later ones*, the series usually running from yellow to orange, through darker tints to brown or black.

Bertrand's studies make it clear that any benzene nucleus with an attached hydroxyl can be acted upon by tyrosinase and converted into melanin pigment. Thus the whole series of compounds in the table given below (and many others besides) can be oxidized to colored compounds. On the other hand, phenylalanine, phenylmethylamine, phenylominoacetic, phenylpropionic and phenylacetic acids, alanin, glycocoll, etc., give no coloration whatever. The size, complexity, and nature of the lateral chain has only a subordinate influence ; if it is not very strongly acid or basic it will not interfere with the oxidation. Thus for example, ethyltyrosin, chloracetyltyrosin, and glycyltyrosin were oxidized with ease. The nature of these side chains does, however, *considerably modify the colors produced* by the oxidation. Neither of the three last named bodies (tyrosin compounds) give a final black color ; they begin with orange or yellow and end with red or mahogany (chocolate ?).

In order to further appreciate something of the variety of color which may arise from a *single chromogen*, and to get an introductory idea of the number and variety of chromogens to be found in the animal body, careful reference to Table I. should be made. *And here it is of the highest importance to see that a single chromogen acted upon by a single enzyme* (so far as all chemical experience has detected) *produces several colors depending upon the degree of oxidation involved.*

In regard to the rate at which these colors appeared the author's statement may be cited that in a 20 per cent. tyrosinase (80 per cent. strong tyrosin) solution, tyrosin developed a rose color in ten minutes and its black color in four to five hours.

TABLE I. (From Bertrand, '08.)

Name of Body.	Colors Produced by Oxidation.
Tyrosin	red grenadine, then inky black.
p-oxyphenylethylamine	red grenadine, then black olivaceous.
p-oxyphenylmethylamine	orange yellow, orange red, maroon.
p-oxyphenylamine	orange, mahogany red, then brown.
p-oxyphenylpropionic acid	orange yellow, grenadine red, brown.
p-oxyphenylacetic "	yellow, orange yellow, then brown.
p-oxybenzoic "	(weak) rose, orange, then yellow.
p-cresol	yellow, orange, then red.
Phenol	yellow, orange, red, then brown.

These are, from our present point of view, the more notable results of Bertrand's investigations of what he calls " the mechanism of melanogenesis." This author does not in any way consider the bearing of his findings on Mendelian descriptions of the mechanism of the origin of melanin *color characters* (neither on any aspect of inheritance). Nor — as previously stated — has anyone, at any time, inquired as to whether the facts obtained in the former sphere are compatible with the assumptions made in the latter. Bertrand's studies had other and quite different objects; these were: First, to learn the degree of specificity of the enzyme tyrosinase; the conclusion here being (p. 387) "that the results speak once more against the principle of very absolute specificity which one nowadays often hears applied to enzyme actions." It will be very well for us to bear in mind this result, since, as we shall see, Mendelian description demands a still higer degree of enzyme specificity than the philosophy of biochemists has yet dreamed of. A second object of his work seems to have been to consider the possibility of identifying certain of these tyrosin bodies by the color reactions they give upon oxidation with tyrosinase. A third purpose of the study was concerned with the causes of the rather wide variations in the elementary composition of different melanins; he believes that the results give some reason for believing that the simplest melanins arise from the oxidation of tyrosin itself, while the more complex ones — those containing sulphur or iron — are formed by the oxidation of less complete products of protein hydrolysis — namely, tyrosin-containing di- or polypeptids.[1] A fourth and final phase of Bertrand's investigation touched upon a hitherto unrecognized, but seemingly possible, mode of union between tyrosin and other amino acids.

* * *

It will now be well to briefly sketch a few facts obtained from the study of abnormal tyrosin metabolism, and from pathological pigmentations (melanin) of the human body, as supplementary to the account of melanogenesis which is given above. These facts will also serve to illustrate the dependence of tyrosin oxidations upon somatic conditions which may be of such a temporary, intermittent, quantitative, or reversible character as to preclude the possibility of accounting for them on the basis of specific, independent transmissions, once for all segregated by the germ cells.

---

[1] In the formation of melanins, condensations as well as oxidations occur, but the former process need not concern us in treating the present theme.

Rather fortunately for the completer view of our present theme, pathologists and clinicians have been frequently confronted with cases of incomplete tyrosin oxidation (alkaptonuria), and unusual and pathological melanin pigmentations (melanotic tumors, Addison's disease, ochronosis) in the human body. These subjects because of their medical bearings have received a very great amount of attention, of accurate and searching study, at the hands of investigators. It seems self-evident that the student of melanogenesis should here find much data to interest him.

In the condition known as "alkaptonuria"[1] the alkapton acids — uroleuic and homogentisic — appear in the urine. The last-named compound represents a stage in the oxidation of tyrosin. The intermediate stages and the chemical structure of these several compounds may be best understood by reference to Table II. Our interest in these early stages of tyrosin oxidation is very great since we know that the same, or similar steps, lead in special cases to the formation of melanin.[2] Neubauer ('08) has very recently determined the exact course of the first four steps of tyrosin oxidation as they occur in the living (human) body. The chemical expression of these stages is given in the table.

Garrod ('02) found that certain individuals, who in their youth excreted urine containing homogentisic and urolencic[3] acids, produced only the former during adult life. Other cases of temporary and intermittent alkaptonuria are known. Here certainly the evidence of our senses is simply that the *power of the organism to oxidize tyrosin compounds is not dependent primarily upon germinal segregations*, but rather upon tissue activities, relations

---

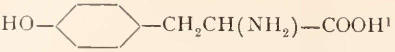
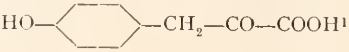
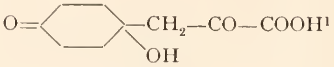
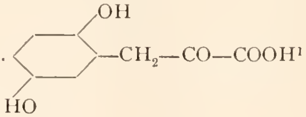
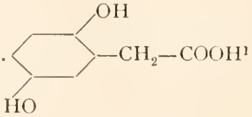
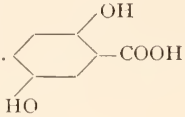
[1] Résumé and literature by Falta, *Biochem. Centralb.*, 3, p. 174, 1904. See also Abderhalden, "Lehrbuch der Physiologischen Chemie," Berlin, 1906, pp. 294–298; and Neubauer ('08), *loc. cit.*

[2] A chemical research directed to the determination of the reasons for some tyrosin oxidation leading to melanin formation, instead of to the usual end-products of oxidation — NH_3, CO_2, H_2O, etc., would be of the greatest interest and help in studies on the physiology, development and heredity of color.

[3] According to Neubauer ('08) this body is not an intermediate step in the oxidation of *tyrosin* to homogentisic acid.

TABLE II.

SHOWING SOME OF THE KNOWN FACTS OF CHEMICAL EXPERIENCE CONCERNING THE SUCCESSION OF OXIDATIONS OF TYROSIN AND CLOSELY RELATED BODIES TO MELANIN PIGMENTS.

Tyrosin =	 [1]	Colorless.
P-oxyphenyl-pyrotartaric acid =	 [1]	“
Chinol =	 [1]	“
Hydrochinon-pyrotartaric acid =	 [1]	“
Homogentisic acid[4] =	 [1]	“
Gentisic acid =		“
Melanogen	( )	“
Melanin	( )	(White) ?[2]
Melanin	( )	Pale yellow.[3]
Melanin	( )	Deep yellow.[3]
Melanin	( )	Red.[3]
Melanin	( )	Brown.[3]
Melanin	$(C_{50}H_{58}N_8SO_{12})$	Black.[3]
Melanin	$(C_{45}H_{78}N_{10}SO_{20})$	(White) ?[2]

and conditions; these may vary or change from year to year—a power of oxidation not possessed by the individual during many early years of life being attained at manhood, or vice versa. Bateson ('02) has seen fit to claim that (p. 133, note) alkaptonuria is the result in inheritance of the union of "two recessives." Garrod has concurred in the view. But in the light of the inconstant and intermittent character of the phenomenon, it seems necessary to draw a directly opposite conclusion.

[1] This according to Neubauer ('08).

[2] See discussion of Spiegler's work ('03), etc., p. 328 of this paper.

[3] These from Gessard ('03) and from Bertrand ('08).

[4] Concerning the formation of homogentisic acid from tyrosin in plant tissues, see Schulze and Castoro, *Zeit. f. Physiol. Chem.*, Bd. 48, p. 396, 1906.

Examples of other temporary or intermittent oxidative powers might be much extended to include cases of glycosuria, cystinuria, purin metabolism, etc. I shall not discuss these cases which have only an indirect bearing on our question of tyrosin oxidation. It is, however, of some interest to state that it has become evident through the work of Abderhalden and Schittenhelm ('05), Garrod and Hurtley ('06), and others, that the body may possess a low oxidizing power for *several different protein constituents at the same time;* as, for example, in some cases of cystinuria, when diamines, tyrosin, lysin, etc., in addition to cystin, pass through the body unoxidized and appear as such in the urine.

The known facts of abnormal pigmentations deserve a larger share of attention than they receive here. They are mentioned chiefly to direct attention to a field of facts that are quite completely ignored in our theories of the heredity of color.

In the condition known as *ochronosis,* certain cartilages (*e. g.*, those of the ear) and connective tissues become pigmented. The work of Albrecht ('02), Osler ('04), Pick ('06), and others, make it certain that ochronosis is a form of melanotic pigmentation, and that it is not uncommonly associated with melanuria, or alkaptonuria and even with the pigmentation of the sclerotics and skin (Osler). Similarly, in *Addison's disease* there is deposited in the skin a pigment which, according to Pforringer ('00) differs from that produced normally only in quantity and not in origin or composition. It is well known too that *nerve lesions* are often accompanied by pathological pigmentation of the skin.[1]

A word in regard to *melanotic tumors.* These are known to occur particularly in white horses. The amount of melanin produced is often very great. Abel and Davis ('96) estimate the melanin of the entire skin of a negro at 1 gram, whereas Nencki and Berdez ('85) found 300 grams of melanin in a sarcomatous liver, and estimate that the entire body contained 500 grams.

These several facts from pathology are significant in that they indicate that *for the building of any melanin at all, the actual local conditions of the organism, or the organ, have a rôle to play that*

---

[1] See résumé by Schmidt, *Ergeb. der Pathol.*, Bd. III., Abt. 1, p. 551, 1896.

is quite out of keeping with any "once for all determination" by the shuffling of color "factors" through the germs.

In this connection attention should be called to the fact that Spiegler ('03) has reported the finding of a *white* melanin in the white hair of horses, and in sheep's wool. It would seem that the melanin isolated by him represents a more advanced stage of oxidation than does black melanin. If this is true it is obviously an important fact. The isolation of a similar pigment from the white hair of *albinos* seems, however, neither to have been sought for, nor found; but it is quite possible that such a pigment also exists in mammalian albinos. There are nevertheless some reasons — chiefly biological rather than chemical — for believing that among birds, at least, a white color exists which represents a less advanced stage of oxidation than that in any other of the melanin color series; in fact, here the "white" seems to be a purely "physical" color. The chromatophores do not develop, and no white pigment is histologically discoverable. Apparently, therefore, the oxidation of tyrosin, etc., is here not carried far enough to produce any color whatever.

The actual facts regarding the "white" pigment or color of birds and mammals are not yet clear. "White" forms at present a most awkward, and at the same time a most interesting gap in our knowledge of the melanin series. The elementary formulas for the black and white pigments given in the table are Spiegler's findings for the white and black hair of the horse. Certain phases of Spiegler's research have been confirmed by Wolff ('04), but the particular facts which we have referred to above have not been reviewed or confirmed.

We are fortunate in having, from physiological experiments with melanin pigments in living animals, some facts which confirm the data from chemistry and pathology regarding the mechanism of melanin production. On this point special attention may be called to the recent work of Gustav Tornier. His work furnishes a splendid view of the *control* of the color of the integument. The colors of Amphibia, from larval stages to old age, were determined at will by controlling the physiological state, particularly the nutrition, of the animals. When the color phenomena observed by Tornier are viewed in the light of the work of Bertrand, it seems certain that in these two sets of phenomena we are really dealing with the same facts.

Tornier found that tadpoles divided into lots for differential feedings gave (1) little or no pigment in the ones fed the minimum, and progressively more pigment as the maximum is approached; (2) *a series of colors: white, yellow, red, gray, black.* Tornier concludes ('07*b*, p. 288): It is possible, therefore, by adjusting the dosage of fleshy food to force the epidermal coloration of *Pelobates* larvæ (he elsewhere describes this as true for other amphibia) into white, yellow (see p. 285), red, gray, black.[1] It will be seen that the types of color and the order of their appearance in the organism — when we put these organisms into such conditions as will force them to do the work of pigment formation (oxidation) in stages — closely follow the lines of our purely chemical experience. Tornier produces entirely comparable effects upon the coloration by two other means, viz.: by removing more or less yolk from the vegetative pole of the egg through an opening made by a needle; or by coagulating *in situ* a part of yolk proteid by the introduction of water; such coagulations of yolk proteid cannot be digested by the developing embryo. The three methods employed all reduce the nutrition of the animals, and produced albinism, erythrinism, blackness, or melanism, depending upon the state of nutrition.

It was further found ('07) that the experiment could be carried out in the *opposite direction* as well; that is to say, the highly fed, black-containing, and black-producing larvæ of large or small size could be made, through a diminution of feeding, to produce a *series of colors in the order of: black, brown, red, gray, white.* Certain observations by Powers ('08) are confirmations of Tornier's [1] results.

Without extending these illustrations it can be stated that if these facts and experiments mean anything they mean that in an animal that produces melaninic color, *there exists all the machinery necessary to produce a series or scale of these colors.* And that what is actually produced is, in several demonstrated instances, *dependent upon the physiological state of the organism.* Or, perhaps, in certain cases it may be possible to say more definitely that the *limiting factor* is none other than the available oxygen or food-supply. I have been able to prove ('08*a*) definitely that in many birds the daily nutritive changes which accompany the low blood-pressures occurring at night influence the *quantity* of melanin produced.

---

[1] The proof that all these shades of color in Tornier's tadpoles were melanins is hardly as complete as is desirable, since lipochromes and traces of guanin are also known to develop normally in late larval and adult stages of these amphibia. Many facts, however, indicate that the colors here described by Tornier are true melanins.

The specific color of an animal then is an index, not of the presence in the germ from which this animal arose, of certain chromogens and specific zymogens, and the absence of a wide series of others ; but, this specific color means that a *process* with a wide range of possibilities, *because of a particular physiological state and environmental conditions* has struck this particular equilibrium. *One and the same organism has within it all that is necessary to move that equilibrium up or down* — taking the red form for example, we can in the words of Tornier "force it to black or to white."

Tornier did not consider the relation of his results to any of the facts of the chemistry of melanin, nor did he consider their bearing on color inheritance. His chief concern has been apparently to establish two points in color physiology; first, the effect of varying degrees of nutrition on the size, shape, color-production, etc., of chromatophores ; and, secondly, a defense of the thesis that the pigment granules of these chromatophores act as reserve food-materials in cases of inanition, etc.

MENDELIAN DESCRIPTION.

It is now possible to consider whether Mendelian interpretation and description is in accord with the facts of color formation. The Mendelian position can be best presented in the words which Cuénot ('03) used in the original formulation and statement of it. I quote practically the whole of his discussion, and ask that it be remembered that it is almost entirely upon this slender basis that the "presence, absence" hypothesis, and consequently a great share of Mendelian nomenclature, rests :

"Again one learns that the authors who have recently studied the origin of melanin pigments, Biedermann, v. Fürth, Schneider and Gessard, state that these pigments result from the action of an oxidizing enzyme (tyrosinase) upon a chromogenic substance; there are good reasons for supposing that things happen similarly in the pigmentation of the skin; there should be, however, in this case, either two different chromogens and only one enzyme, or only one chromogen and two enzymes, the one for the blackish pigment and the other for the yellow pigment. We adopt provisionally, for convenience of language, this latter hypothesis.

"The germ plasma of a gray mouse should contain potentially the three substances which, by their reciprocal reactions later produce the deposition of pigment in the hair; and doubtless these three substances are contained in the potential state within many of the material particles of the germ plasma (representative particles or qualitative substances of the egg — mnémons). In a gray mouse (black and yellow pigmented) there are three mnémons, one for the chromogen and two for the two ferments; in a black mouse there are only two mnémons, one for the chromogen and another for the formative enzyme of black pigment.

"In regard to albinos, all is explained if we admit that their germ plasma contains only the mnémons of the enzymes, that of the chromogen being totally absent. With these conditions, colored hair cannot be formed in albinos, since one of the substances indispensable to the reaction is absent, but one easily understands that the albino will transmit to its progeny either the mnémons for the two enzymes, or one mnémon only, if it possesses but one."

The Mendelians have one further point to confirm the faith that is in them. Soon after the appearance of the paper by Cuénot, Durham undertook to find whether in the skins of black, chocolate, yellow and albino mammals there is the appropriate enzyme in each for the production of its particular color — when this acts upon a tyrosin solution. Only a short preliminary statement ('04) of the results has appeared; and although positive results were reported for the black, chocolate and yellow pigments, it is evident that from no point of view can these results be regarded as satisfactory; particularly because the extracts used by her are stated to have had a *reddish* color before adding the tyrosin; an adequate account of the history or nature of color changes in such a solution seems hardly possible since as Table I. shows much of melanin production results in colors which are paler than red and the origin of all these, and to some extent the final color, would be obscured by the initial presence of red. Miss Durham was unable to state exactly what the extracts of the albino skins were capable of doing, but thought they probably contained no such enzymes.

The factor hypothesis of Castle avoids some of the pitfalls of the earlier theory, but seems to rest on essentially the same base. It is necessary to examine in detail the statements and conclusions in Cuénot's paper.

Cuénot says: "There should be, however, in this case either two different chromogens and only one enzyme, or only one chromogen and two enzymes, the one for the blackish, the other for the yellow pigment." The facts of the origin of melanin do not substantiate Cuénot's hypothesis because the colors do not form on this plan; one and the same chromogen is known to form yellow and black; one and the same ferment — tyrosinase — is known to produce both yellow and black from the same chromogen.

According to Cuénot: "The germ plasma of the gray mouse (black and yellow pigment) should contain potentially the three substances which, by their reciprocal reactions, later produce the deposition of pigment in the hair; and doubtless these three substances are contained in the potential state within many of the material particles of the germ plasma (representative particles, mnémons, etc.)." But three such substances are not required for the production of black and yellow; these two color compounds are known to be but different stages of oxidation of the same substance. Furthermore, the locating of all the factors which determine the development of these colors in the germ plasm does not reckon with the facts of color physiology already cited.

Continuing, Cuénot says: "In a gray mouse (black and yellow pigmented) there are three mnémons, one for the chromogen, and two for the two enzymes; in a black mouse there are only two mnémons, one for the chromogen and another for the formative enzyme of black pigment." Statements made above furnish a sufficient refutation of this conception of Cuénot's.

Cuénot's concluding statement: "In regard to albinos all is explained if we admit that the germ-plasma contains only mnémons of the enzymes, that of the chromogen being totally absent. With these conditions, colored hair cannot be formed in albinos, since one of the substances indispensable to the reaction is absent, but one easily understands that the albino will transmit to its progeny either the mnémons for the two enzymes, or one mnémon only if it possesses but one." To this statement must be opposed first the opinion of Durham that the skins of the albino mammals studied by her contained no tyrosin-oxidizing enzymes; a second much more weighty and conclusive objection is that the absence of melanin chromogens in albinos is practically inconceivable. It is now certain that tyrosin and its many related compounds are such chromogens, and that these compounds have a distribution in the universe almost or quite co-extensive with protoplasm itself. *The postulation of the formation of chromogen-containing, and non-chromogen-containing gametes is therefore reduced to an absurdity. It is moreover quite certain that the food of the albino mouse must daily bring it quantities of chromogens, even if such could have been excluded from the germ cells. There is no doubt and no middle ground here. Cuénot's conception fails completely.*

Space does not permit a discussion of the facts and interpretations of the inheritance of coat colors of mice since the work of Cuénot; a subject which has been investigated or discussed by many workers, notably by Bateson, Allen, Cuénot, Morgan, Wilson, Castle and Durham. A detailed consideration of these results is omitted also because the facts are well known and do not belong here. The behavior of the color yellow first reported by Cuénot ('05) is, however, of such unusual interest as to deserve special mention. It was found that yellow is "dominant" in mice, whereas elsewhere in animals it is usually "recessive" to black; this is an unexpected result from the Mendelian standpoint, and the difficulties which it presents have called forth several highly complex, supplementary Mendelian hypotheses. It seems not to have occurred to anyone that yellow may be a *blend* formed from the union of other colors, *e. g.*, between albino and black. A glance at our scale of colors (p. 326) suggests that such may be a true, and, indeed, the truest conception of a color blend; and an examination of the experimental results gives considerable support to this view. Castle ('06) reports that he secured yellow mice from three sources, viz.: Y♂ × Ch ♀, A × B, A × Ch. It will be observed that each of these crosses has one color (yellow, chocolate, black, albino) *more* oxidized than yellow, and one *less* oxidized than yellow (see Table II.); that is, the yellow produced in these cases is apparently a blend.[1] The general fact of the unstable (heterozygous) character of all yellow mice is, quite possibly, evidence of the same kind.

Heretofore a blend, say between white and black, has been considered a mixture of these two colors, a spotted animal, a form in which black was diminished, etc., but a little reflection upon facts already stated concerning the nature of these color characters reveals *very distinct colors as none the less very distinct blends!*

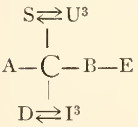
At present the *biological* data are wanting to quantitatively seriate all of the several colors; but there is apparently enough data to warrant the definite statement that *yellow mice* are forms with the power to oxidize tyrosin compounds to an *intermediate point.* Thus the biological data again parallel chemical experience.

Cuénot, Bateson and others "explain" color inheritance on the assumption that "recessives" lack altogether the *factors* of color production; but Castle has convincingly argued that this cannot be true, because in such forms *small amounts* of pigment actually form, etc. Castle tried to explain these phenomena upon the supposition that all of the factors may be present, but that "the presence of one character often inhibits the activity of another," *i. e.*, upon grounds of activity and latency. I would urge that we are now quite ready to take the next step, which seems to lie in the opposite direction, and say that we have to do neither with absent factors nor with the inhibition of present factors; that in gametic unions we deal not at all with "factor" particles, but merely mix, and amalgamate to various degrees, powers of tyrosin oxidation; and the conditions supplied by the differentiation of tissues and organs, together with environmental conditions external and internal, supply whatever else is concerned in color production.[1]

---

[1] Cuénot says of his yellow mice that they contain numerous unfixable variations, not hereditary, ranging from a clear orange yellow to a sooty or grayish yellow, not very different from the color of gray mice. Does this look like purity of gametes, or a wide range of blending, which?

In his work on mice Castle ('06) shows conclusively that in these forms he is not dealing with the *total absence of a character*, even in some cases where this seems apparent; he there states that the *purity of gametes* does not exist, and further, "no more does the *purity of factors* exist." It would seem that this paper by Castle is one of the best, if not the best document extant to convince that no such things as *factors* exist. Yet, quite recently,[2] Castle has carried the factor hypothesis to its highest state of complexity, to what is apparently its logical conclusion, if any "particle" basis whatever could be granted for hereditary processes. Castle pictures his conception of the factors in melanin color inheritance and their relations in the following diagram, modelled after a chemical formula:



$$\begin{array}{c} \mathrm{S} \rightleftarrows \mathrm{U}[3] \\ | \\ \mathrm{A} - \mathrm{C} - \mathrm{B} - \mathrm{E} \\ | \\ \mathrm{D} \rightleftarrows \mathrm{I}[3] \end{array}$$

C = Color, or chromogen factor.
A = Agouti factor.
B = Black "
D = Dilution "

E = Extension factor.
I = Intensity "
U = Uniformity "
S = Spotted "

This visualizes for us the body of factors which are to be shuffled in the germs and "determine" the colors of the progeny. From what has been said it is obvious how far this conception leads us astray; because this complex Medelian interpretation and description of color inheritance leads us away from a simple series of color developments due to the difference in degree to which one substance may be oxidized.

The placing of the "uniformity-spotted," "intensity-dilution," and "extension" factors in these germs is a virtual surrender of the whole theory of discontinuous variation; and in reality puts the Mendelian who accepts this terminology in no position to deny the development of the melanin series on a basis of closely graduated powers of tyrosin oxidation, and so without any basis on particles or factors, whatever. It is, moreover, as plain as it is certain, that this *degree of oxidizing power* covers several of these factors (A, B, E, I, D) which are thus reduced to one; while the kernel of this formula the C, or chromogen, goes out entirely as a factor, *i. e.*, something capable of being shuffled in the germs — since as we have pointed out, such a chromogen is universal in protoplasm.

---

[1] The many and accumulating additions, qualifications, "contaminations," "latencies," etc., that have been attached to Mendel's ('65) original conception of dominance and recessiveness, or to Cuénot's ('03) presence and absence of a factor hypothesis, are but so many direct admissions that the "purity of gametes" conception is an error; they are but so many secondary and tertiary hypotheses of completer preformation made to bolster up a primal preformation hypothesis. Incidentally, they give present-day students the opportunity to see the child of Weismannism recapitulate the developmental history of the parent.

[2] Darwinian lecture, Baltimore, January 1, 1909.

[3] Either one of the pair may be present (or active).

Discussion.

If the later oxidations — those which produce color — of the tyrosin compounds are each individually controlled by a separate, specific enzyme, why are not the several earlier oxidations of the same compounds similarly conditioned? If assumption will give us the whole series of tyrosin oxidations only on condition of their production by means of separate and distinct enzymes, why should it hesitate to put the whole vast array of oxidations of all aromatic, or even of all organic compounds on a similar basis — a separate and specific enzyme, separately heritable, for each step in oxidation? This is certainly not true.

It is impossible at present to announce the limits to the specificity of enzymes, and of the oxidases generally; but it can be said that it is pretty generally conceded that the oxidases present less specificity than do the digestive enzymes (see Wilcock, '06). That which weighs most heavily against the Mendelian assumption of a high specificity of tyrosin oxidizing enzymes is, however, the result of Bertrand's special study of tyrosinase which indicates no such specificity (quoted p. 324 of this paper).

But granted the greatest possible specificity of these enzymes, the Mendelian description of color inheritance becomes even more untenable; for Gessard and Bertrand have shown that "black" is the end-result of a series of successive oxidations and this final color can be attained only by having *all* of the intermediate stages actually attained. This means that the animal that transmits the enzyme for black, *i. e.*, produces black colored offspring, must *at the same time* transmit also the enzyme for *brown, chocolate, red, yellow, etc.* (more accurately, an enzyme for each step of oxidation from tyrosin to black melanin), *without the absence of a single one.* If there be introduced here the primal Mendelian conception of the freedom of *each of these* factors to be distributed in gametogenesis according to the laws of chance, how often then may we expect pure-bred black parents to produce black offspring?[1]

There are reasons, derived from our general knowledge of oxidizing enzymes, why this assumption of high tyrosinase specificity is highly improbable and some evidence against this position has been cited from Gessard and Bertrand; a direct refutation of it is furnished by the experiments of Tornier. He showed that he could take animals which would have, according to Mendelian assumption, the enzyme for "black," and make them produce any one of three or four of the less oxidized members of the color series; these same forms could again under other conditions be made to produce the more highly oxidized black, etc. Obviously, the presence of a black-producing enzyme did not determine the color here; but conditions of life did so determine. The further assumption of inhibiting factors ready at all points of color-production to account for lower grades of color formation, and all other secondary assumptions to support the primary one are clearly unnecessary, and since a clearer, saner interpretation is possible they need not be considered.

---

[1] This would be the usual or expected type of specificity if such thing should exist. I call attention to its implications merely to forestall any further thought regarding its possibility. The sort of specificity of enzymes that has thus far been assumed by the Mendelians has, however, been of a different sort; namely, that for the production of each color only one enzyme is necessary, but the enzyme which produces any particular color is specifically different from those which produce other colors; the case is not really different from an assumption that pepsin is specifically different in different, but closely related races and varieties. Unlike the case cited above, here each germ possesses only one or two zymogens, each capable of supervising the complete production of some one color, and therefore all the offspring could (in contrast to above) be provided with color. The only evidence that has been adduced for this sort of specificity is the rather incomplete and unsatisfactory results of Miss Durham already cited. All else has been mere assumption on the part of the Mendelians. But this type of specificity becomes a very unusual and extraordinary thing as soon as we find that all of the colors form in a continuous oxidation series. To cover this fact the assumption is obligatory that in melanin production, six, ten or a dozen tyrosin oxidizing enzymes are concerned; that these are all able to take the first steps of tyrosin oxidation; that they are differentiated merely by their "strength," that is, the extent to which they can carry the oxidations.

The doctrine of numerous specific enzymes,[1] then, goes the way of the doctrine of specific *chromogens*, which is so decisively settled by the work of Bertrand.[2] Remembering that the Mendelian description (by implication) of what is happening on the (color-producing) surface of the body in late stages of development is thus completely awry, we may not be surprised to find that their assumptions regarding conditions in the germ are in a similarly contradictory tangle.

Our present knowledge permits neither the realization nor the imagination of a "color factor" in the germ; not even in a simple form; much less does it grant us the very composite and elaborate picture presented by Castle.

The "production of color" is a special manifestation, in rather restricted regions of an organism, of a *general power to oxidize organic compounds, possessed, presumably, by all parts of the germ cell* from which the organism arose. With the development of the body, the specialization of tissues, there arise very new environments for the oxidative processes, producing localized changes and variations in this power. That this is so is evidenced by the fact that the living substance of the various body regions oxidizes fats, sugars, and proteids with unequal ease. There can be, moreover, scarcely a doubt that certain of these regions, owing to new structure, new environment, new conditions, are able to oxidize *different protein substances* with variable ease, and to a variable extent, and even in a different way.

When one has grasped the nature of the process by which melanin color characters are formed, there is as little necessity or truth in assuming that the germ cells contain representatives or determiners to correspond to a particular color, as there is in assuming that the vapors of the Gulf contain determiners for the depth, or distribution, of the mantle of snow which they are to form on the northern hills. There exists, to be sure, a relation between the vapors and the heaps of snow, between the egg and the definitive character — but the one is not the other, does not *contain* the other.

---

[1] Mendelians must, however, accept the specificity or non-specificity of enzymes in the production of color (they must have some sort of representative particle in the germ) and either choice leads, when critically examined, to the unqualified refutation of some of their fundamental conceptions and interpretations of heredity. The established fact that each of the melanin colors represents but a point in a line — a line which records the continuity of a continuous oxidation process — is the fact that strikes hard at the very basis of Mendelian interpretation and description.

[2] Table I. shows, for example, that at least seven of the nine compounds represented are capable of producing the colors yellow and red.

In accounting for melanin color characters, I would maintain that — granted the continuance of other life and developmental processes — we can account for all the major happenings of color development and inheritance with extremely little of assumption. It is *known* that one oxidase is concerned. I think we need make use of but one. It is *known* that the germ possesses actively the power to oxidize amino acids. I think we need make use of nothing in the germ than just this power. It is *known* that the protoplasm of different species, of different tissues, of different parts of a cell, possess different powers of oxidizing protein bodies. It is no tremendous assumption that germ cells are not freaks of nature in this respect, and that they too have different powers of tyrosin oxidation. The fate of color characters then is bound up (1) in the union of these particular powers of the two germ cells; (2) in the origin (through other outside developmental processess) of favorable and unfavorable regions for tyrosin oxidations; and (3) in environmental conditions.

Let us now for a moment return to the matter of color-blends. The data given furnish a nearer view — practically a new conception — of the nature of color-blends in inheritance; if the position stated in regard to these color-blends is correct a little thought will convince that at the same time new light is furnished on *alternative* color inheritance; and particularly on *what is happening* in the case of the so-called alternative (Mendelian) inheritance of a color character. It can be said definitely in such a cross that it is the *power to oxidize tyrosin compounds* (a power which I believe, by no stretch of imagination, needs to be, or can be, represented by a particle in the germ, but by a general property of the protoplasm of germ cells and of tissue cells) *that is transmitted* and that here this power of *one* of the gametes *is continued into*[1] the zygote without being very *appreciably* increased or diminished.

---

[1] Tyrosinase has recently been found in the *eggs* of cephalopods by Weindl ('07). Similarly, several enzymes have been isolated from germ cells in recent years; in none of these cases can we for a moment suppose that these enzymes or zymogens existed as *a* particle in any one, or in each, of the chromosomes. I would, however, by no means have it inferred that I consider the presence, quantity, etc., of tyrosinase

It may be, however, that more than one (almost certainly several for yellow) oxidation stage of a tyrosin compound presents only the one color black. It could conceivably (see scale of colors, p. 326) happen, therefore, that a certain black (low stage of oxidation) × light yellow = yellow (dominant?); but another black (highly oxidized) × yellow = black (dominant?); *and yet each of these might be true blends*, *i. e.*, attain to an exact intermediate stage of oxidation to the two forms crossed. Some cases of supposed dominance of color may be therefore in reality true examples of blended inheritance.

In describing color inheritance I believe that less violence is done to the known facts of color formation, and at the same time a sounder view of developmental and hereditary processes is maintained, if it be said — without delimiting terminology, and without putting a single thing into the germ except what every one knows is there, and there in the form which is stated for it — that in the union of germ cells derived from two pure color varieties, each cell brings with it a power of oxidizing tyrosin compounds, and that the union of the pair of cells may give one or more of the following results:

1. The tyrosin oxidizing power of the male cell is established (*a*) at once, or (*b*) in the next generation or later, throughout the fertilized ovum and its derivatives.

2. The tyrosin oxidizing power of the female cell is so established. (In neither of these cases do we need to postulate the continued existence of a subdued — recessive — factor or representative.) These would be so-called dominants.

3. The oxidizing power resulting from the union is somewhat more, or somewhat less, than that of either of the gametes.

---

in the egg as an index, measure, or determiner of the tyrosin oxidation powers of the adult. Secrets of protoplasmic differentiation, zymogenesis, stereochemistry, and catalytic action, all block for the present the tracing or predication of any such relations.

Undoubtedly enzymes have a very considerable importance in development. By focusing attention too closely upon them it is possible, however, to underestimate the importance of other, and even of related phenomena in which the *possibility* of "inherited specificity" is entirely eliminated. I refer particularly to the "autocatalysis" of such substances as oils studied by Guenthe ('07), Mathews, Walker and the writer ('08*b*), and others.

4. The result of the union is a blend; *i. e.*, an oxidizing power intermediate to that of the two gametes.

Numbers 1 and 2 represent colors at points of fairly fixed color equilibrium, as proved by the fact that individuals, varieties and species tend to stop color formation at those points; and most of the offspring of such hybrids may reasonably be expected to breed true with reference to this character, because of such stable equilibrium. Categories 3 and 4 often represent, on the other hand, colors at points of unfixed equilibrium; stages in the oxidation of tyrosin are not easily held at these points; that such points of unstable equilibrium arise in the chemical building of melanin, as elsewhere, is practically certain; this unstable condition is followed by an immediate tendency — in the second (next) generation usually — to shift to one or both of the stable points represented by the male and female condition, or to a new point.[1]

The above considerations seem to be of far-reaching applicability. They are, I think, rigorously consistent with what we know of the oxidation process, and with the various facts of melanogenesis; whereas Mendelian interpretation is consistent with neither. How much of the totality of color-inheritance is thus brought under one point of view will be at once appreciated by naturalists; while in striking contrast is the very small fraction of such inheritance that can be brought into the Mendelian system, even with all its elaborations.

In following out the implications of our conception of the state in which these color characters exist in the germ, it may be said that hybrid offspring possessing a color of easy, fixed equilibrium, mated with similar forms may usually be expected to breed true (that is, to continue this oxidizing power into their germ cells) with respect to this character. If mated, however, with another variety possessing some very different character, let us say size, which is also in very stable, fixed equilibrium, it seems quite conceivable — almost a necessity — that each variety should at least sometimes be able to force its stable character (if these do not both rest directly upon the same process, *e. g.*, tyrosin oxidation) practically unchanged into a new combination and produce a new form — an animal with the *color* of one parent and the *size* of the other.

---

[1] These four types give nothing of *qualitativeness* nor of *discontinuity;* we have to deal absolutely in these initial stages with *quantity*, degree or pitch of oxidation power, and the gaps which we find in the end-result of the development of the color characters are but the cumulative, final expressions of different degrees of oxidation power, and of the fact that certain stages of oxidation are more stable — in firmer equilibrium — than others.

This clearly brings us face to face with something resembling unit characters and particulate inheritance. I can see no reason, from studies on the nature of color formation, and from the necessary deductions as to the way in which color must be transmitted, to doubt the possibility or the probability of the formation of races with new characters, or rather a race with a new combination of old characters; and with some such newly combined characters in very stable equilibrium, *i. e.*, breeding true. In fact, it is the recognition of the state in which our color character exists in the germ, that is, as a given pitch of power for oxidizing tyrosin compounds, and this not latent but active in the germ as later, that enables us to view the mechanism by which such a result is brought about.

The demonstration of the existence of such combinations of characters is, I believe, the supreme contribution of Mendelism to our knowledge of heredity. Phenomena of dominance, of segregation (?) and proportion, are but minor and special manifestations of a process much more important, general and inclusive; and which general process is, in color inheritance at any rate, the propagation and occasional quantitative modification (four types above) of oxidizing powers, with their more or less constancy of expression through settling into points of easy or fixed oxidizing equilibrium.

Our view does not, however, allow the acceptance of the unit character hypothesis without very considerable and rather radical modification. The prevailing idea among Mendelian workers has been essentially that each character, each recognizable differentiation, each member of a group of factors that forms a character, is quite separate and capable of being shuffled in the germs, and of independent appearance in the zygote. Now, as already noted, experience with the melanins drives home the point that a long series of very distinct characters have not each even *one* representative, but *all* together have one basis in the germ — a power to oxidize tyrosin compounds, and this capable of close and continuous gradations. Many other characters, moreover, may also be closely connected genetically with the tyrosin oxidizing power of the organism. This being true, it is easily seen that what has been quite generally taken to be a "unit character" among colors cannot be in fact a "unit" of *modification.* All tyrosin oxidations, and some others as well, may be, and probably are, modified simultaneously and in corresponding manner.

The conception of "units of modification," then, which we adopt as an apparent logical necessity cannot regard as "units" the things that have been called unit characters in the past; quite certainly some unsound criteria, a false interpretation, a misleading nomenclature, and some observed facts are all back of the old conception as it has been applied to color behavior. We need not be surprised if it be found to embrace very little truth. To determine what a real "unit of modification" is, would seem to be no easy matter. Present Mendelian methods will doubtless contribute something, though not nearly all, to the complete story. For if — and there is little doubt — other definitive characters of the soma are likewise present in the germ only as varying "strengths" of rather general powers or processes, these same powers will exercise influence directly and indirectly, and to greater or less extent, in many and diverging directions during development; so that a "unit of modification" in inheritance would, in the broadest sense, include all such effects (there would probably be found all gradations of such effects). Obviously, therefore, *individual characters are by no means units of inheritance or modification.* And in any exhaustive search for such influence or modification it is quite possible that vision will sometimes be forced to cover the whole field from antibodies and immunity to size and color; from the grossest structural modification to the most delicate functional idiosyncrasy.

The question will be asked — how may those who reject the Mendelian interpretation based on representative particles, account for the segregation and proportions observed in Mendelian behavior? To undertake a discussion of this point is obviously to leave the province of fact, with which the body of this paper deals to enter the realm of assumption and hypothesis. The fact, that in these pages we have been able to get what we are inclined to consider the clearest view that we have at present as to the state in which a character or set of characters exists in the germ, perhaps furnishes a reason why some statement may here be made in regard to the *possible mechanism of segregation.*

It has already been stated that to us the phenomena of dominance and segregation are only minor, surface, and incidental phenomena of heredity;[1] the really important Mendelian contribution being that certain different characters (such as have, according to my belief, different rather general processes as a basis) of different races may be combined to form new fixed races. The establishment of this last-named fact has been most commonly considered by Mendelians as, on the one hand, a consequence of the laws of dominance and segregation, and, on the other hand, as a strong argument for a "representative particle" basis for these two sets of phenomena. When, however, we learn that a certain character has no other existence in the germ than a rather general protoplasmic power, the "mnémon" conception fails completely and with it the supposed mechanism of its segregation — *i. e.*, the shuffling of mnémons in the reduction divisions of the chromosomes.

How then on my view of the basis of color inheritance may the segregation and proportions which result in Mendelian behavior be accounted for? I may say at once that I do not know; but in this respect I consider myself hardly worse off than the wisest Mendelian. My *supposition*, however, would put less faith than is theirs in the behavior of chromosomes during the maturation divisions. It may for the present be assumed, and this is, I think, all that is really demanded in accounting for the differences in end-result in color development and inheritance — that the germ cells vary in "strength," *i. e.*, in such general powers as assimilation, growth, oxidation, etc. (and this proposition is not all assumption); this general difference of "strength" of germ cells may (or may not) arise during the maturation divisions; one or more of the four cells receiving in some species, though not in all, a type of protoplasm in better or worse condition than the others — influenced, for example, by more or less yolk; yolk more or less affected by previous nuclear and cytoplasmic contact; by variable distribution of the protoplasm of the astrosphere; by variable admixtures of nuclear (not necessarily chromatin) and cytoplasmic matter; by receiving more or fewer entire chromosomes, etc. (Chromatin is well known to be a very reactive substance and we may very well believe that wide variations of it in amount influence the vigor or intensity of vital processes occurring in a cell and in its derivatives; but in this respect chromatin is not unique, the other variables just mentioned doubtless do the same.) Any or all of these things will influence the development of a character only by serving to strengthen or weaken some *process* which underlies its formation.[1]

---

[1] It would seem that instead of viewing the real and entire sea of heredity, learning of its intimate nature, searching its boundaries, sounding its depths, too often Mendelians have — to indulge a figure — focused their visual instruments upon an optical section lying perhaps a few meters *above* sea-level. Here, occasionally, beautiful and regular phenomena come into sight; but for the most part the field is blank. At times great ocean swells pass in fine order and precision, permitting the observer to predict quite well some of the attributes of the next undulation and the time of its appearance; again, bits of spray or foam or mist may sometimes come into view, and the seeming disconnectedness of it all permits the be-focused onlooker to name and classify — and wonder. All — while the great ocean of heredity with its perfect continuities, its essential oneness, its inclusiveness, lies in unseen constancy and majesty beneath.

Such general differences of germ cells as arise from the several possible causes mentioned above would conceivably tend to affect the strength of such a general process as oxidation — such as produces scales of color. From the nature of these differences it will be seen that the growth and maturation stages may occasionally among organisms — constantly perhaps in some forms — furnish the conditions for the production of four sister sperm cells of unequal strength, one or two may be especially favorably equipped by the distribution of such materials, while one or two are left poorer than the others.[1] A similar conception may be applied to the ova. Then upon the union of male and female cell, two oxidizing powers of equal or unequal rank, of higher or of lower degree, meet. A stable (pure breed) high equilibrium results in, say one of four; a stable (pure breed) lower equilibrium results in another; while in the other two perfect equilibrium is not at once attained.

---

[1] It is perhaps well in our estimation of the basis for segregation of color characters, which rest directly on an oxidation process, to call attention to the special significance of the centrosome and cytaster. Considerable evidence is adduced by Mathews ('07) to show that the centrosome is the reduction center of the cell. If this should prove true, very obviously our estimation of the oxidation powers of the cell would be better treated in relation to this body than to any other in the cell. It cannot be too much emphasized, however, that upon this view the centrosome and the sphere is but a *region* where intense reduction occurs; the intensity grading off from centriole to (presumably) the periphery of the cell; and this region should be thought of as an expression of general powers of the whole cell.

Here is then a *possible* picture of the basis of Mendelian segregation and proportion, but without recourse to hypothetical "particles" or to immutable and immortal factors. An apparently very specific end-result of an oxidation would be traceable in the germ only in the strength or pitch of a general vital process, and not at all in mnémons or representative particles packed with unthinkable precision, order and potentiality into (presumably) the chromosomes. But the above is a possible picture only; and it is not here my purpose to furnish nor to defend at length a possible or probable theory of the mechanism of heredity. The material in hand lends itself first of all to the demonstration of the *impossibility* of many Mendelian views.

The nature of present Mendelian interpretation and description inextricably commits to the "doctrine of particles" in the germ and elsewhere. It demands a "morphological basis" in the germ for the minutest phase (factor) of a definitive character. It is essentially a morphological conception with but a trace of functional feature. Although heredity is quite surely a functional process of major complexity, it may be recalled that the primary and fundamental Mendelian conception of this process utilizes not a single finding of the science of biochemistry; that the only physiological fact utilized is the one of certain occasionally observed segregation behavior which is exhibited in the end-results of varietal[2] or specific character formation; such segregation, by

---

[1] We know that in the corresponding divisions of *ovogenesis* that extremely disproportionate distribution of yolk and cytoplasm occurs quite constantly. We may believe that the laws which there give rise to the *extreme* differences between polar body and oöcyte are not *everywhere else*, and *completely*, unoperative; they may be operative — though in a much less pronounced degree — in providing different *mature* ova, and different sperms with varying amounts of the materials mentioned above.

[2] De Vries ('01, '05) has asserted that only varietal, not specific, differences exhibit Mendelian heredity; this statement is not accepted by Bateson ('06), and is controverted by still other workers.

inference, arising from the temporary mixture (heterozygote), or failure to mix (homozygote) in the gamete, of something, no one knew what — but which has been generally conceived of as some sort of "particles." (In *later* additions to, and *special* applications of the Mendelian conception, certain other biochemical and physiological facts have, of course, been considered.)

This is precisely why present Mendelian interpretation and description of heredity is a bar to the progress of studies in inheritance and development; with an eye seeing only *particles*, and a speech only symbolizing them, there is no such thing as the study of a *process* possible.

The conception that organic color has at its basis not rigid, immortal particles, but yielding, equilibrium-seeking powers, or strengths of processes, makes the infinite variety of colors in organisms intelligible. If, on the other hand, particles, and a mechanism for their continual segregation and propagation pure were in reality at the basis of color inheritance, we should rather expect uniformity, not the actual diversity, to be the dominant feature of organic coloration. Indeed, a modification of the strength of many organic processes (and so of color formation) would be a necessary accompaniment — a result, even if not a cause — of that "transformation of organs" which has been the very labor of phylogenetic development. The atrophy, superior development and transformation of organs are certainly efficient factors in such modification, for it is a physiological fact of common experience that many, or most, of the vital processes are not equally strong or pronounced in all of the organs of the same organism; and that many metabolic processes of the body are dependent upon special organs for their highest expression, for the completest manifestation of their power.

Let me not be understood to say that our knowledge of the development of melanin color characters is complete. There is much yet to be learned. But the significant thing about it is that we now know *so much* of the mechanism of the building of these characters in comparison with what we know of a similar nature in non-color characters. It has been possible, I think, to show by means of what we know of the genesis of these color characters that the Mendelian description — of color inheritance at least — has strayed very wide of the facts; it has put factors in the germ cells that it is now quite certainly our privilege to remove; it has declared discontinuity where there is now proved continuity; it has postulated preformation where there is now evident epigenesis.

Is it too much to expect that the further application of such tests as the one here presented in outline for the melanin colors will in the end remove *many* of the Mendelian "factors" from the germ cells? That many of their "characters" will come to rest on a more proximate basis; will be known to have their "determination" and origin in very general germinal powers, and in somatic conditions obtaining previous to, or at the time of, their development? Will not other Mendelian discontinuities then begin to disclose gradations, and other qualitative differences then appear more and more as quantitative sequences?

Laboratories of Experimental Therapeutics and Zoölogy,
The University of Chicago,
January, 1909.

LITERATURE CITED.

Abel, J., and Davis, W.
'96 The Pigments of the Negro's Skin and Hair. Jour. Exp. Med., I., p. 361.

Abderhalden, E., and Schittenhelm, A.
'05 Ausscheidung von tyrosin und leucin in einem falle von cystinurie. Zeit. f. Physiol. Chem., XLV., p. 468.

Albrecht, H.
'02 Ueber ochronose. Zeit. f. Heilk. Path., Abt. 23, p. 366.

Bateson, W., and Sanders, Miss E. R.
'02 Report to the Evolution Committee.

Bateson, W.
'06 Progress of Genetics.

Bertrand, G.
'96 Sur une nouvelle oxydase ou ferment soluble oxydant, d'origine végetalé. Bull. Soc. Chim., 3d Ser., T. XV., p. 791, and C. R. Ac. Sc., T. 122, p. 1215.
'03 Sur les relations du chromogène surrénal avec la tyrosine. Compt. rend. Ac. Sc., T. 138.

Bertrand, G., and Mutermilch, W.
'07 Recherches sur le mode de coloration du pain bis. Ann. Inst. Pasteur, T. 21, p. 833; also C. R. Ac. Sc. and Bull. Soc. Chim., '07.

Bertrand, G.
'08 Recherches sur la mélanogenésis: Action de la Tyrosinase sur la Tyrosine. Ann. de Inst. Pasteur, Vol. 22, No. 5, p. 381.

Biedermann, W.
'98 Beiträge zur vergleichenden physiologie der verdauung. I. Die verdauung der larve von Tenebrio molitor. Pflüger's Archiv, 72, p. 105.

Castle, W. E.

'06 Mice and Gametic Purity. Science, N. S., 24, No. 609, p. 275.

Chittenden, R. H., and Albro, Alice H.

'99 Formation of Melanins or Melanin-like Pigments from Proteid Substances. Amer. Jour. Phys., II., p. 291.

Cuénot, L.

'03 L'hérédité de la pigmentation chez les souris. Arch. Zool. Exp., 4, No. 3, p. 33.

'05 Les races pures et leurs combinaisons chez les souris. Ibid., 4, T. 3, Notes et revue, p. 123.

DeVries, H.

'01 Die mutationstheorie. Leipzig ('01 and '03).

'05 Species and Varieties, Their Origin by Mutation. Chicago.

Dewitz, J.

'02 Recherches experimentales sur la métamorphose des insectes. Compt. rend., T. 54, p. 44.

Duccheschi, V.

'01 Sulla nature delle melanine e di alcune sostanze ad esse affini. Rendic. della R. Acad. dei Lincei, Cl. di Scienzi fisiche (5), Vol. X., Fasc. 5, Seduta del 3 Marzo.

Durham, F.

'04 On the Presence of Tyrosinase in the Skins of Some Pigmented Vertebrates. Prelim. Note. Proc. Roy. Soc. London, Vol. 74.

Garrod, A. E.

'02 The Incidence of Alkaptonuria : A Study in Chemical Individuality. Lancet (2), p. 1616.

Garrod, A. E., and Hurtley, W. H.

'06 Concerning Cystinuria. Jour. Physiol., Vol. 34, p. 217.

Gessard, M. C.

'01 Études sur la tyrosinase. Ann. Inst. Pasteur, T. XV., p. 593.

'02a Tyrosinase animale. Compt. rend. soc. biol., T. 54, p. 1304.

'02b Antityrosinase animale. Ibid., p. 1398.

'03a Sur les oxydases des Seiches. Compt. rend. Ac. Sc., T. 136, p. 631.

'03b Sur la formation du pigment mélanique dans les tumeurs du cheval. Compt. rend. soc. biol., T. 136, p. 1086.

'03c Ueber das Pigment des Nebennieren. Compt. rend., T. 138, p. 586.

'03d Sur les réactions colorées consécutives à l'action de la tyrosinase. Compt. rend., T. 138, p. 774.

Gmelin, L.

'94 Ueber das schwarze Pigment des Auges. Beiträge z. Chemie u. Physik von Schweigger, Bd. X.

Gonnermann, M.

'00 Homogentisinsäure die farbebedingende substanz dunkler Rübensäfte. Pflüger's Archiv, Bd. 82.

Hlasiwetz, H., and Habermann, J.

'73 Ueber die proteinstoffe. Liebig's Annalen, 169, p. 150.

Hopkins, F. G., and Cole, S. W.

'01, '03 A Contribution to the Chemistry of Proteids. Part I. and II. Journ. Phys., 27.

Landolt, H.

'99 Ueber das Melanin der Augenhäute. Zeit. f. phys. Chem., Bd. 28.

Lepinois, M. E.

'99 Études sur le chromogène des capsules surrénales et sur l'origene de la coloration rouge que ces glandes prennant au contact de l'air. Compt. rend. Soc. Biol., Vol. 51, p. 315.

Mathews, A. P.

'07 A Contribution to the Chemistry of Cell Division, Maturation, and Fertilization. Am. Jour. Phys., Vol. 18, p. 89.

Mendel, G.

'65 Versuche ueber Pflanzen-Hybriden. Abhandl. Naturf. Ver. in Brünn, IV. Also English translation by Bateson, Jour. Roy. Hort. Soc., XXVI., 1901.

Nencki, M.

'95 Zur Kenntniss der pankreatischen Verdauungsprodukte der Enweisskörper. Ber. Dtsch. Chem. Ges., Bd. 28.

Nencki, M., and Berdez, J.

'85 Ueber die Farbstoffe der melanotischen Sarkome. Arch. Exp. Path. und Pharm., Vol. XX., p. 346.

Neubauer, O.

'08 Ueber den Abbau der Animosäuren im Gesunden und kranken Organismus. Habitationsschrift-Vogel, Leipzig.

Osler, W.

'04 Ochronosis: The Pigmentation of Cartilages, Sclerotics and Skin in Alkaptonuria. Lancet (1), p. 10.

Pförringer.

'00 Zur entstehung des hautpigments bei Morbus Addison. Cent. f. Allg. Path. (11).

Powers, J. H.

'08 Morphological Variation and its Causes in Amblystoma tigrinum. Studies from Zoöl. Lab. of Nebraska, No. 71.

Pick, L.

'06 Ueber die ochronosis. Ber. klin. Wochenschr. (43), p. 478.

Ravenna, E.

'03 Beiträge zur Histogenese der Melanotischen Hautgeschwülste. Virchow's Arch., Bd. 171.

Riddle, O.

'08a The Genesis of Fault-bars in Feathers and the Cause of Alternation of Light and Dark Fundamental Bars. Biol. Bull., XIV., p. 328.

'08b (With A. P. Mathews and S. Walker.) The Spontaneous Oxidation of Some Cell Constituents. Abst. in Jour. Biol. Chem., IV., June, p. xx.

Samuely, F.

'02 Ueber die aus Eiweiss hervorgehenden Melanine. Hofmeister's Beitrage, II.

Schmiedeberg, O.

'97 Ueber Elementarformeln einiger Eiweisskörper und ueber die Zusammensetzung und die Natur der Melanine. Arch. Path. u. Pharm., Bd. 39.

Spiegler, E.

'03 Ueber das Haarpigment. Hofmeister's Beiträge, Vol. IV.

Stadelmann, E.

'90 Ueber das beim Tiefen Zerfall der Eiweisskörper entstehende Proteinochromogen den die Bromreaction gebenden Körper. Zeit. f. Biol., Vol. 26.

Tornier, G.
'07a Experimentelles ueber Erythrose und Albinismus der Kriechtierhaut. Sitz'-ber. Ges. Nat. Freunde, Berlin, p. 71.
'07b Nachweis ueber das Entstehen von Albinismus, Melanismus und Neotemie bei Fröschen. Zool. Anz., Bd. 32, p. 284.
'08a Ueber eine Albinotische Ringelnatter und ihre Entstehen. Sitz'ber. Ges. Nat. Freunde, Berlin, No. 8, p. 196.
'08b Vorläufiges ueber experimentell erzielten Hautalbinismus bei Axolotl-Larven. Sitz'ber. Ges. Nat. Freunde, Berlin, No. 3.
'08c Vorläufiges ueber das Entstehen der Goldfischvassen. Ibid., No. 3.

von Fürth, O.
'99 Ueber die Einwirkung von Salpetersäure auf Eiweisstoffe. Habilitato-Schrift., Strassburg.
'04 Physiologische und chemische Untersuchungen ueber melanotische Pigmente. Centralb. f. All. Path., Bd. XV. (older literature).

von Fürth, O., und Schneider, H.
'01 Ueber tierische Tyrosinasen und ihre Beziehungen zur Pigmentbildung. Hofmeister's Beiträge, Bd. I.

Weindl, Theod.
'07 Pigmententstehung auf Grund vorgebildeter Tyrosinasen. Arch. Ent'mech., Bd. 23, pp. 632.

Wilcock, E. G.
'06 The Action of Radium Rays on Tyrosinase. Journ. Physiol., Vol. 34, p. 207.

Wolff, H.
'04 Zur kenntnis der Melanotischen Pigmente. Hofmeister's Beiträge, V., p. 476.

MBL/WHOI LIBRARY
WH 17JM C